LOW-GRADE INFLAMMATION, IMMUNE CAPACITY, AND CANCER

By
Sarah Alice Davidovics

A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, MD
October, 2014

Abstract

Inflammation is a well-established etiological factor in carcinogenesis. The immune system may also have the capacity to identify and clear malignant cells in a process known as tumor immunosurveillance. The objective of this dissertation is to examine these roles of host immunity in carcinogenesis in immunocompetent adults. Specifically, we quantified the risk of cancer incidence and mortality by circulating, pre-diagnostic levels of the white blood cell (WBC) subtypes, neutrophils, lymphocytes, monocytes, eosinophils, and basophils, and cytomegalovirus (CMV) IgG titer in two longitudinal cohorts, the Atherosclerosis Risk in Communities (ARIC), 1987-2008, and the third National Health and Nutrition Examination Survey (NHANES III), 1988-2011. Multivariate Cox analyses were used to estimate the hazard ratios (HR) and 95% confidence intervals (CI) of cancer incidence and mortality by levels of immune markers. High neutrophil count was associated with an increased risk of total (HR: 1.11, 95% CI: 1.00, 1.25) and lung (HR: 1.59, 95% CI: 1.12, 2.26) cancer incidence, and total (HR: 1.44, 95% CI: 1.22, 1.72), lung (HR: 1.66, 95% CI: 1.18, 2.33) and breast (HR: 2.09, 95% CI: 1.10, 3.97) cancer mortality. Among men, high lymphocyte count was associated with a reduced risk of cancer incidence, after excluding prostate cancer (HR: 0.75, 95% CI: 0.62, 0.91), and an increased risk of prostate cancer incidence (HR: 1.31, 95% CI: 1.03, 1.66). Among women, lymphocyte count was positively associated with cancer mortality (HR: 1.40, 95% CI: 1.07, 1.82). The presence of basophils in circulation was associated with a reduced risk of cancer incidence (HR: 0.93, 95% CI: 0.85, 1.01) and mortality (HR: 0.87, 95% CI: 0.76, 1.00). Adjustment for the other WBC subtypes did not appreciably alter these estimates. No associations were found for monocyte or

eosinophil counts. Lastly, high CMV IgG antibody titer was associated with increased cancer mortality in black CMV seropositive persons (HR: 1.38, 95% CI: 1.02, 1.89), while no association was present in whites or Mexican Americans. Our findings of an association between circulating pre-diagnostic levels of neutrophils, lymphocytes, basophils, and CMV IgG and cancer incidence and mortality support a role for low-grade inflammation and subclinical immune suppression in carcinogenesis.

Thesis Readers

Kala Visvanathan, MBBS, FRACP, MHS (Advisor)
Associate Professor in Epidemiology and Oncology

Mei-Cheng Wang, PhD
Professor of Biostatistics

Ana Navas-Acien, MD, PhD
Associate Professor of Environmental Health Sciences, Epidemiology

Bryan Lau, PhD, ScM, MHS
Assistant Professor of Epidemiology

Paul Strickland, PhD, MSc (alternate)
Professor of Environmental Health Sciences

Katherine Clegg Smith, PhD, MA (alternate)
Associate Professor of Health, Behavior and Society

Corinne Joshu, PhD, MPH, MA (alternate)
Assistant Professor of Epidemiology

Acknowledgments

Thank you to my dissertation advisor and mentor, Dr. Kala Visvanathan. Dr. Visvanathan has guided my development as an epidemiologist for the past seven years with encouragement, patience, and integrity. She has been incredibly generous with her time and this work is a testament to her commitment to the research and her role as a mentor.

Thank you to the faculty in the department of epidemiology at the Bloomberg School of Public Health for helping to train me and for sharing your expertise to develop and enhance this project. In particular, I would like to thank my thesis committee, Dr. Elizabeth Platz and Dr. Patti Gravitt, and my dissertation readers, Dr. Mei-Cheng Wang, Dr. Ana Navas-Acien, and Dr. Bryan Lau.

I know I would never have completed this goal without the boundless love and support of my parents and sister. I constantly seek to emulate your determination, work ethic, honesty, loyalty, faith, intellectual curiosity, and humor. And, to my husband, Natan, and our baby, Amitai: you are my constant inspirations and have made my life so full and happy. The best part of this entire endeavor was that it led me to you, my two loves.

Table of Contents

List of Tables

List of Figures

Chapter 1. Introduction

Overview and Specific Aims

This overall objective of this dissertation is to explore the roles of low-grade inflammation and immune suppression in the context of tumor initiation, promotion and progression. Inflammation is a well-established factor in carcinogenesis, with known effects throughout the multi-stage pathway, from tumor initiation to metastasis (1-4). In prospective epidemiological studies, elevated levels of systemic markers of inflammation have been associated with an increased risk of cancer incidence and mortality (5-16). There is also strong evidence primarily from mice models supporting a role for the host immune response in blocking carcinogenesis through the identification and clearance of premalignant and malignant cells (17). The effectiveness of this anti-tumoral mechanism, known as tumor immunosurveillance, may to relate to the capacity of the immune system to mount a response.

The burden of cancer risk attributable to subclinical immune dysfunction may be significant. As many as 20% of cancers have been linked to chronic infection (18). Additionally, subclinical inflammation and immunosuppression may party mediate the carcinogenic effects of many common exposures, including obesity, smoking, and age (4, 19). In a review of the emerging field of cancer immuno-epidemiology, the need for prospective studies evaluating the association between immune biomarkers and cancer risk was highlighted (20). Such studies have the potential to clarify important etiologic pathways and to identify opportunities for prevention. **In this dissertation, we utilized the immune makers, white blood cell (WBC) subtype count and cytomegalovirus (CMV) IgG antibody titer in a series of specific aims:**

Specific Aim 1. To prospectively evaluate the association between the WBC subtypes, neutrophils, lymphocytes, monocytes, eosinophils and basophils, and cancer incidence (1987-2006) and mortality (1987-2008) in the Atherosclerosis Risk in Communities (ARIC) study. In men and women with total WBC counts within the normal reference range, we estimated the risk of total and site-specific cancer incidence and mortality by counts of the WBC subtypes using multivariable models, including mutual adjustment for each of the WBC subtypes.

Specific Aim 2. To explore effect modification by sex, race, cigarette smoking status, and the genetic markers, percent European ancestry and the Duffy null polymorphism (neutrophils only), on the prospective association between the WBC subtypes, neutrophils, lymphocytes, monocytes, eosinophils and basophils, and cancer incidence (1987-2006) and mortality (1987-2008) in the ARIC study. We examined potential effect modifiers known to modulate the host immune response. In analyses of neutrophil count, effect modification by percent European ancestry and the Duffy null polymorphism were investigated in order to explore the implications of racial differences in absolute neutrophil count.

Specific Aim 3. To prospectively examine the association between levels of CMV IgG antibody titer and cancer-specific mortality in the third National Health and Nutrition Examination Survey (NHANES III) study, 1988-2011. We estimated the independent association between levels of CMV IgG antibody titer and cancer mortality

among CMV seropositive individuals, ages 40 to 70 years. We also evaluated effect modification by sex, race and cigarette smoking status.

Background

Inflammation and cancer

A role for inflammation in the etiology and progression of cancer is well established (1-3). Based on data from in vitro and in vivo studies, chronic inflammation may initiate carcinogenesis by inducing mutagenesis directly, via the production of radicals, or indirectly, by inactivating critical DNA repair proteins and other mechanisms, including epigenetic changes (4, 21-23). Inflammation may also promote tumor growth and metastasis through mechanisms primarily directed by the tumor itself. Inflammatory cells are an important component of the tumor microenvironment, which can be co-opted to promote tumor cell proliferation and angiogenesis, inhibit tumor cell death, and induce immune suppression to subvert the host immune response against tumors (1-4, 24, 25).

Findings from epidemiological studies support an etiological role for inflammation in carcinogenesis. Numerous studies have reported a positive association between chronic benign inflammatory conditions, such as pancreatitis and inflammatory bowel disease (IBD), and subsequent cancer risk (26-28). Furthermore, among IBD patients, the severity of inflammation was found to be an independent risk factor for cancer incidence (26, 29, 30). Additionally, as many as 20% of cancers have been linked to chronic infections (18). It is widely hypothesized that the association between infectious agents, including persistent Heliobacter pylori and hepatitis B and C, and cancer is partly mediated by inflammation (3, 31, 32). Among individuals with no underlying immune conditions, inflammation is also a proposed mechanism underlying the association between common exposures, such as age, cigarette smoke, and obesity, and cancer (4,

19). Furthermore, long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) has been shown to reduce the incidence and mortality of certain cancers, including colorectal, breast, prostate, lung, stomach, esophageal, and pancreatic cancers (33-37).

Prospective epidemiological studies have reported a modest positive association between levels of circulating inflammatory markers and the risk of total cancer incidence and mortality (5, 7-9, 11-13, 15); A positive association has also been found for certain site-specific cancers, including lung (7, 8, 10, 13-15), colorectal (6, 7, 10, 14, 16, 38), and breast (7, 10) cancers. In these studies, a significant association was present after adjustment for many potential confounders, including age, sex, race and cigarette smoking status. Additionally, a positive association persisted after excluding incident cancers and cancer deaths within the first several years of follow-up to account for the presence of subclinical disease (6, 9-11, 38).

In the majority of the aforementioned studies, systemic inflammation was quantified using levels of circulating total WBC count and/or C-reactive protein (CRP), which are non-specific inflammatory markers (7-16, 38). A few other studies have examined the association between select cytokines (i.e., interleukin-6 and tumor necrosis factor-α) (8, 39) and other acute phase reactants (i.e., erythrocyte sedimentation rate, serum globulin, and fibrinogen) (5, 6), and cancer incidence and mortality. Thus, the epidemiologic literature is limited with regard to characterizing and quantifying the impact of low-grade inflammation on tumor initiation, promotion and progression (20). This limitation is

particularly profound as in vivo models evaluating the effects of inflammation in the early stages of carcinogenesis are largely missing (4).

Tumor immunosurveillance

The tumor immunosurveillance concept, first described by Burnet and Thomas in the late 1950's (40, 41), posits a role for the host immune system in the identification and clearance of premalignant and malignant cells. More recently, experimental studies in murine models have provided convincing support for this anti-tumoral response, leading to a resurgence of interest in this theory (13, 36, 37). In these studies, the capacity of certain immune cells, mediators and modulators to mount a response is inversely associated with the development and progression of cancer. These findings have been reported for components of both the innate and adaptive immune systems. Additionally, in in vitro and in vivo studies, tumor cells that escape elimination by the host immune system have been shown to have reduced immunogenicity (42). This mechanism of tumor survival and growth, termed tumor immunoselection, has been proposed to be the seventh hallmark of cancer (43).

In epidemiological studies, an increased risk of cancer in severely immunosuppressed patients has been reported in several studies. Among transplant patients, for example, immunosuppression is the strongest, known risk factor for cancer (40-42). Kidney transplant patients have a two to five-fold increased risk of de novo cancer compared to the age-matched general population (43-46), and in studies comparing renal transplant

patients to controls on kidney waiting lists and dialysis patients, this increased risk of cancer persists (47, 48).

In transplant patients, an increased incidence of cancers of primarily viral origin is consistently reported (41, 43-47, 49, 50), while the association with common epithelial cancers remains unclear (47, 50-53). Similarly, patients diagnosed with human immunodeficiency virus (HIV) or acquired immune deficiency syndrome (AIDS) are at increased risk of developing cancers of viral origin, but have been shown to have a slightly reduced risk of breast, ovarian, colon, and prostate cancer (54, 55). Importantly, however, the interpretation of these findings is challenging given the diverse effects of immunomodulating medications and underlying co-morbid conditions on both immune functions and cancer risk in the severely immunocompromised. Additionally, differential screening and medical surveillance in the immunosuppressed may account for the decreased risk of certain cancers (50).

Few epidemiological studies have examined the relationship between host immune capacity and cancer in immunocompetent individuals (20). In numerous studies, a history of allergies has been associated with a reduced risk of cancer, particularly pancreatic cancer and gliomas (44, 45). The mechanism underlying this inverse association has been hypothesized to be the tumor immunosurveillance response, given that allergies indicate a state of heightened immunity, however, there is limited data directly linking immune components of the allergic response to cancer risk (45, 46). Additional support for the tumor immunosurveillance response comes from cross-sectional studies in which natural

killer (NK) cell-mediated cytotoxicity and lymphocyte proliferation, measures of immune capacity, were suppressed in cancer-free individuals with a familial history of cancer compared to controls with no family history of cancer (56-60). To date, only one prospective study has directly explored the association between immune capacity and cancer risk. In this study of 3,625 healthy Japanese men and women, high NK cytotoxic activity was associated with a 41% reduction in cancer incidence after 11 years of follow-up (47). Notably, the paucity of epidemiological studies directly exploring the tumor immunosurveillance response may be partly due to the lack of cohort studies with available measures of immune capacity *and* prospectively collected information on cancer outcomes.

Immune biomarkers and cancer

In a review of the emerging field of cancer immuno-epidemiology, Nakachi et al. (20) highlight the need to explore additional, novel immune biomarkers in order to better characterize low-grade inflammation and immune capacity in the context of tumor development and progression. In response to this knowledge gap, we identified several markers that may help to elucidate the role of the host immune system in the carcinogenesis pathway. These markers include the WBC subtypes, neutrophils, lymphocytes, monocytes, eosinphils and basophils, and CMV IgG antibody titer.

WBC subtype counts

In prospective studies, total WBC count, a nonspecific marker of systemic inflammation, has been consistently, positively associated with cancer risk and cancer-specific mortality

(5, 6, 9-12), with the exception of a few studies (63-65). Risk estimates comparing the highest and lowest quartile of WBC count, within the normal reference range, were 1.1 to 1.4 for non-lung cancer incidence and 1.65 to 1.75 for cancer-specific mortality, after multivariable-adjustment of potential confounders such as smoking (9-12).

Total WBC count consists of five diverse cell types, which have differential roles in the host immune response (3, 66, 67). In healthy adults, neutrophils account for 50 to 70% of the total WBC count while lymphocytes (30%), monocytes (5%), eosinophils (1-3%) and basophils (0 to 3%) constitute the remaining cell types. Neutrophils and monocytes are activated early in the acute and innate inflammatory responses, particularly in response to bacterial infection, whereas T and B lymphocytes are involved predominantly with adaptive immune responses, and eosinophils and basophils are closely linked to allergic responses (48). Data from laboratory studies suggest that the WBC subtypes may also have specific roles in carcinogenesis, with the capacity to exert both pro- and anti-tumoral effects (24). However, to date, only three epidemiological studies have explored the association between pre-diagnostic WBC subtype counts and subsequent cancer development and progression.

In one of these studies, conducted in a large U.S. based cohort of adult men and women (N= 10,675), the highest tertile of eosinophil count was associated with a 42% reduced risk of colorectal cancer incidence, compared to the lowest tertile, in multivariable-adjusted models including age, race, sex, education level, cigarette smoking status and total WBC count. Notably, this association held after excluding cancer cases within the

first five years of follow-up to account for the presence of subclinical disease and after excluding persons with eosinophil counts >0.3 $x10^9$ cells/L, as levels above this limit may indicate an acute response. Furthermore, this association seemed to be specific to colorectal cancer as no association was found between eosinophil count and lung or breast cancer incidence (49).

Additionally, in a community-based cohort study conducted in Denmark, Sajadieh et al (50) examined the association between counts of circulating WBC subtypes and cancer incidence among 669 middle aged and elderly men and women without prevalent cancer or cardiovascular disease (CVD) at baseline. In the main analyses, elevated monocyte count (>0.60 $x10^9$ cells/L) was associated with a two-fold increased risk of total cancer incidence after adjustment for sex, age, smoking, level of CRP, alcohol usage, and heart rate variability. In age- and sex-adjusted models only, no association was found between the other WBC subtypes and cancer incidence.

While these results are intriguing, they must be interpreted in light of several limitations. First, meaningful cut-offs of WBC subtype count were not evaluated. Specifically, the range of monocyte count in the highest quintile was 0.60 to 3.9 $x10^9$ cells/L, while the normal reference range for monocyte count in adults is 0.2 to 0.95 $x10^9$ cells/L (48). Thus, it is unclear if the increased risk of cancer incidence associated with the highest quintile of monocyte count reflects variations within this normal range or is largely driven by persons with abnormal monocyte counts. Furthermore, information on the range and distribution of the other WBC subtypes was not provided. Second, this study

was limited by a relatively short follow-up period of 6.3 years and the possibility of reverse causality was not evaluated. Third, in analyses of neutrophils, lymphocytes, basophils and eosinophils, multivariable-adjusted models were not conducted and effect modification was not assessed. Fourth, due to the limited number of outcomes, cancer mortality and cancer incidence site-specific analyses were not possible.

Additionally, in the Sajadieh et al study (50), racial differences could not be addressed given the racial composition of the cohort. Race may be a particularly relevant factor when studying neutrophils as racial differences in the levels of circulating neutrophil count have been widely reported, with significantly lower levels in healthy black adults compared to whites (51-53). This difference has been linked to a single nucleotide polymorphsim (SNP) in the Duffy antigen receptor for chemokines (DARC) (54). It is plausible that neutrophil function also differs by race, and that this may have implications for carcinogenesis. However, this hypothesis has not been previously investigated.

Finally, in a study of nearly 10,000 elderly Korean men and women, the highest quartile of monocyte count was significantly associated with increased cancer mortality during a total of 6.5 years of follow-up, based on a non-adjusted comparison of survival curves (55). In this study, there was no association between the other WBC subtype counts and cancer mortality.

CMV IgG antibody titer

Cytomegalovirus (CMV) is an endemic beta herpes virus with seroprevalence estimates in the U.S. ranging from 36.6% in 6-11 year olds to 90.8% of adults ages 80 years and older (56). Following infection, CMV cannot be cleared by the host immune system resulting in an extensive and life-long immune response to ensure viral latency. In immunocompetent individuals, a low level, baseline CMV IgG antibody titer is established post infection. Among immunocompetent persons, CMV IgG levels are hypothesized to vary depending on the frequency and intensity of subclinical CMV reactivation (57-59).

Cytomegalovirus (CMV) is postulated to be an etiological factor in carcinogenesis, based on laboratory studies. Specifically, in in vivo and in vitro studies, CMV has been shown to alter the expression of factors regulating cell survival, replication, motility and adhesion (60-63). Cytomegalovirus (CMV) may also promote carcinogenesis through its effects on host immunity, via the impairment cellular immunity by inducing T-cell anergy (64, 65) and the deregulation the host inflammatory response (60). A pro-tumoral role for CMV is also supported by clinical studies showing CMV viral products in tumor samples of the colon, cervix, breast and prostate, but not in the surrounding tissue (66-69) However, these findings are not consistent across all studies of tissue samples (70) and, in a cross-sectional study of South African men, circulating CMV IgG level did not vary in cancer patients compared to non-cancer controls (71).

Notably, it is also possible that CMV reactivation is an epiphenomenon of immune dysfunction, rather than a causal factor (60). Cytomegalovirus reactivation is dependent on host immune function (72, 73), including subclinical changes in immune capacity and inflammation. Indeed, increased CMV reactivation has been found to occur in immunosuppressed populations such as the elderly (74), astronauts in space (75), and in persons experiencing stress or loneliness (76). As such, CMV IgG antibody titers may be an informative marker of immune function, generally (57, 59, 77), and tumor immunosurveillance, specifically, in addition to its potential causal role in carcinogenesis.

Few prospective studies have explored the relationship between CMV infection and the risk of cancer incidence and mortality. In two large studies, CMV seropositivity was not associated with an increased risk of prostate cancer incidence (78, 79). Additionally, the presence of CMV viral products in benign tissue samples was not associated with subsequent cancer risk (80). Cytomegalovirus (CMV) seropositivity was also not associated with breast cancer risk in a cohort of young women, however, among CMV seropositive women, elevated CMV IgG conferred an increased risk of invasive breast cancer (81) and a significant increase in CMV IgG antibodies was found to precede diagnosis (82).

To our knowledge, only one earlier study has evaluated the relationship between CMV infection and cancer mortality. In a large cohort based in the United Kingdom, the highest tertile of CMV IgG antibodies was positively associated with all cause mortality, as

compared to a CMV seronegative group, and a suggestive, but not statistically significant, association was observed for cancer mortality and CVD mortality (58). However, in analyses restricted to CMV seropositive individuals, there was no association by titer level (58). Notably, a major limitation of this study is the inclusion of persons with a history of cancer at baseline. Thus, the temporality of the reported associations is questionable. Additionally, these study findings may not be generalizable to U.S. populations, where the prevalence of CMV infection is significantly higher (56, 58) and the patterns of seroprevalence may also differ.

Study populations

To explore the role of WBC subtype counts and CMV IgG levels in tumor initiation, promotion and progression, we used data from two large, well-established cohort studies based in the United States.

ARIC

The ARIC study is an ongoing prospective cohort initiated in 1987 to investigate the etiology of atherosclerosis and its sequelae. In total, 15,792 men and women, ages 45 to 64 years, were recruited to ARIC from four U.S. communities, Forsyth County, NC; Jackson, MS; Washington County, MD; and suburban Minneapolis, MN. Probability sampling was employed to ensure that the study population is representative of the source communities, with the exception of Jackson, MS, where only African Americans were included and Forsyth County, NC, where African Americans were oversampled. Cohort

participants underwent a clinical exam at baseline (1987-1989) and at three follow-up visits conducted triennially (1990-1992, 1993-1995 and 1996-1998).

At enrollment and each of the follow-up study visits, ARIC technicians collected fasting blood samples and other clinical data relating to cardiovascular risk. Participants also reported information on sociodemographic factors, medical history, reproductive history, physical activity, alcohol and tobacco use, and other lifestyle behaviors via standardized questionnaires. Demographic, health information and aspirin use was updated annually by telephone interview and participant information was linked to cancer registry databases and the national death index (NDI) through 2006 and 2008, respectively.

NHANES III

NHANES III is a cross-sectional study of non-institutionalized, civilian, United States citizens aged two months and older. This study was conducted between 1988 and 1994 and was designed to provide nationally representative estimates of health conditions. To achieve this, a complex, multi-stage, clustered, stratified sampling approach was undertaken, with oversampling of African Americans, Mexican Americans, children (< 5 years) and the elderly (> 60 years) to obtain reliable statistics for these groups. Sample weights were generated for each person to account for this non-random sampling approach and non-response. These weights, based on U.S. Census Bureau population estimates at the time, assign the number of people in the U.S. population represented by each participant.

Participation in NHANES III consisted of two phases: (1) an extensive interview in the participant's residence and (2) a visit to the Mobile Examination Center (MEC) where blood was collected and a series of clinical examinations and five automated questionnaires were conducted. Detailed sociodemographic and health information was collected, including cancer and immune-related risk factors such as age, race/ethnicity, sex, income, education level, BMI, personal history of cancer, prior diagnoses of autoimmune or inflammatory disease, recent infections and allergies reactions, physical activity, tobacco and alcohol consumption, menopausal status and hormone use. Additionally, participant information was linked to the NDI through 2011.

Outline of dissertation

This dissertation is comprised of five research chapters (chapters 2-6). Our study of the prospective associations between WBC subtype counts and cancer incidence and mortality is divided into four chapters, with separate sections for neutrophils (chapter 2), lymphocytes (chapter 3), basophils (chapter 4), and monocytes (chapter 5). In chapter 6, we explore the association between CMV IgG levels and subsequent cancer mortality among CMV seropositive adults. This dissertation concludes with a summary of our findings, implications for cancer prevention research and for the clinical setting, and directions for future epidemiological studies.

References

1. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell. 2005;7:211-7.
2. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357:539-45.
3. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420:860-7.
4. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140:883-99.
5. Godsland IF, North BV, Johnston DG. Simple indices of inflammation as predictors of death from cancer or cardiovascular disease in a prospective cohort after two decades of follow-up. QJM. 2010.
6. Prizment AE, Anderson KE, Visvanathan K, Folsom AR. Association of inflammatory markers with colorectal cancer incidence in the atherosclerosis risk in communities study. Cancer Epidemiol Biomarkers Prev. 2011;20:297-307.
7. Heikkila K, Harris R, Lowe G, Rumley A, Yarnell J, Gallacher J, et al. Associations of circulating C-reactive protein and interleukin-6 with cancer risk: findings from two prospective cohorts and a meta-analysis. Cancer Causes Control. 2009;20:15-26.
8. Il'yasova D, Colbert LH, Harris TB, Newman AB, Bauer DC, Satterfield S, et al. Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. Cancer Epidemiol Biomarkers Prev. 2005;14:2413-8.
9. Erlinger TP, Muntner P, Helzlsouer KJ. WBC count and the risk of cancer mortality in a national sample of U.S. adults: results from the Second National Health and Nutrition Examination Survey mortality study. Cancer Epidemiol Biomarkers Prev. 2004;13:1052-6.
10. Margolis KL, Rodabough RJ, Thomson CA, Lopez AM, McTiernan A. Prospective study of leukocyte count as a predictor of incident breast, colorectal, endometrial, and lung cancer and mortality in postmenopausal women. Arch Intern Med. 2007;167:1837-44.
11. Van Hemelrijck M, Holmberg L, Garmo H, Hammar N, Walldius G, Binda E, et al. Association between levels of C-reactive protein and leukocytes and cancer: three repeated measurements in the Swedish AMORIS study. Cancer Epidemiol Biomarkers Prev. 2011;20:428-37.
12. Shankar A, Wang JJ, Rochtchina E, Yu MC, Kefford R, Mitchell P. Association between circulating white blood cell count and cancer mortality: a population-based cohort study. Arch Intern Med. 2006;166:188-94.
13. Allin KH, Bojesen SE, Nordestgaard BG. Baseline C-reactive protein is associated with incident cancer and survival in patients with cancer. J Clin Oncol. 2009;27:2217-24.
14. Siemes C, Visser LE, Coebergh JW, Splinter TA, Witteman JC, Uitterlinden AG, et al. C-reactive protein levels, variation in the C-reactive protein gene, and cancer risk: the Rotterdam Study. J Clin Oncol. 2006;24:5216-22.
15. Trichopoulos D, Psaltopoulou T, Orfanos P, Trichopoulou A, Boffetta P. Plasma C-reactive protein and risk of cancer: a prospective study from Greece. Cancer Epidemiol Biomarkers Prev. 2006;15:381-4.

16. Gunter MJ, Stolzenberg-Solomon R, Cross AJ, Leitzmann MF, Weinstein S, Wood RJ, et al. A prospective study of serum C-reactive protein and colorectal cancer risk in men. Cancer Res. 2006;66:2483-7.
17. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Adv Immunol. 2006;90:1-50.
18. Aggarwal BB, Vijayalekshmi RV, Sung B. Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. Clin Cancer Res. 2009;15:425-30.
19. Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, et al. Cancer is a preventable disease that requires major lifestyle changes. Pharm Res. 2008;25:2097-116.
20. Nakachi K, Hayashi T, Imai K, Kusunoki Y. Perspectives on cancer immuno-epidemiology. Cancer Sci. 2004;95:921-9.
21. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis. 2009;30:1073-81.
22. Cooper CS, Foster CS. Concepts of epigenetics in prostate cancer development. Br J Cancer. 2009;100:240-5.
23. Kraus S, Arber N. Inflammation and colorectal cancer. Curr Opin Pharmacol. 2009;9:405-10.
24. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454:436-44.
25. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer. 2006;6:24-37.
26. Bernstein CN, Blanchard JF, Kliewer E, Wajda A. Cancer risk in patients with inflammatory bowel disease: a population-based study. Cancer. 2001;91:854-62.
27. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut. 2001;48:526-35.
28. Lowenfels AB, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, et al. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. N Engl J Med. 1993;328:1433-7.
29. Rutter M, Saunders B, Wilkinson K, Rumbles S, Schofield G, Kamm M, et al. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. Gastroenterology. 2004;126:451-9.
30. Gupta RB, Harpaz N, Itzkowitz S, Hossain S, Matula S, Kornbluth A, et al. Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. Gastroenterology. 2007;133:1099-105; quiz 340-1.
31. Moss SF, Blaser MJ. Mechanisms of disease: Inflammation and the origins of cancer. Nat Clin Pract Oncol. 2005;2:90-7; quiz 1 p following 113.
32. Ernst PB, Gold BD. The disease spectrum of Helicobacter pylori: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. Annu Rev Microbiol. 2000;54:615-40.
33. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet. 2011;377:31-41.

34. Gonzalez-Perez A, Garcia Rodriguez LA, Lopez-Ridaura R. Effects of non-steroidal anti-inflammatory drugs on cancer sites other than the colon and rectum: a meta-analysis. BMC Cancer. 2003;3:28.
35. Garcia Rodriguez LA, Huerta-Alvarez C. Reduced incidence of colorectal adenoma among long-term users of nonsteroidal antiinflammatory drugs: a pooled analysis of published studies and a new population-based study. Epidemiology. 2000;11:376-81.
36. Elwood PC, Gallagher AM, Duthie GG, Mur LA, Morgan G. Aspirin, salicylates, and cancer. Lancet. 2009;373:1301-9.
37. Takkouche B, Regueira-Mendez C, Etminan M. Breast cancer and use of nonsteroidal anti-inflammatory drugs: a meta-analysis. J Natl Cancer Inst. 2008;100:1439-47.
38. Erlinger TP, Platz EA, Rifai N, Helzlsouer KJ. C-reactive protein and the risk of incident colorectal cancer. JAMA. 2004;291:585-90.
39. Stark JR, Li H, Kraft P, Kurth T, Giovannucci EL, Stampfer MJ, et al. Circulating prediagnostic interleukin-6 and C-reactive protein and prostate cancer incidence and mortality. Int J Cancer. 2009;124:2683-9.
40. Burnet M. Cancer: a biological approach. III. Viruses associated with neoplastic conditions. IV. Practical applications. Br Med J. 1957;1:841-7.
41. Thomas L. Discussion. In: Lawrence HS, editor. Cellular and Humoral Aspects of the Hypersensitive States. New York: Hoeber-Harpter; 1959. p. 529-32.
42. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity. 2004;21:137-48.
43. Zitvogel L, Tesniere A, Kroemer G. Cancer despite immunosurveillance: immunoselection and immunosubversion. Nat Rev Immunol. 2006;6:715-27.
44. Turner MC. Epidemiology: allergy history, IgE, and cancer. Cancer Immunol Immunother. 2012;61:1493-510.
45. Turner MC, Chen Y, Krewski D, Ghadirian P. An overview of the association between allergy and cancer. Int J Cancer. 2006;118:3124-32.
46. Wang H, Rothenbacher D, Low M, Stegmaier C, Brenner H, Diepgen TL. Atopic diseases, immunoglobulin E and risk of cancer of the prostate, breast, lung and colorectum. Int J Cancer. 2006;119:695-701.
47. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet. 2000;356:1795-9.
48. Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J. Harrison's Principles of Internal Medicine. 18 ed: McGraw-Hill; 2011.
49. Prizment AE, Anderson KE, Visvanathan K, Folsom AR. Inverse association of eosinophil count with colorectal cancer incidence: atherosclerosis risk in communities study. Cancer Epidemiol Biomarkers Prev. 2011;20:1861-4.
50. Sajadieh A, Mouridsen MR, Selmer C, Intzilakis T, Nielsen OW, Haugaard SB. Monocyte number associated with incident cancer and mortality in middle-aged and elderly community-dwelling Danes. Eur J Cancer. 2011;47:2015-22.
51. Haddy TB, Rana SR, Castro O. Benign ethnic neutropenia: what is a normal absolute neutrophil count? J Lab Clin Med. 1999;133:15-22.

52. Freedman DS, Gates L, Flanders WD, Van Assendelft OW, Barboriak JJ, Joesoef MR, et al. Black/white differences in leukocyte subpopulations in men. Int J Epidemiol. 1997;26:757-64.
53. Lim EM, Cembrowski G, Cembrowski M, Clarke G. Race-specific WBC and neutrophil count reference intervals. Int J Lab Hematol. 2010;32:590-7.
54. Reich D, Nalls MA, Kao WH, Akylbekova EL, Tandon A, Patterson N, et al. Reduced neutrophil count in people of African descent is due to a regulatory variant in the Duffy antigen receptor for chemokines gene. PLoS Genet. 2009;5:e1000360.
55. Kim KI, Lee J, Heo NJ, Kim S, Chin HJ, Na KY, et al. Differential white blood cell count and all-cause mortality in the Korean elderly. Exp Gerontol. 2013;48:103-8.
56. Staras SA, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ. Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. Clin Infect Dis. 2006;43:1143-51.
57. Dowd JB, Aiello AE. Socioeconomic differentials in immune response. Epidemiology. 2009;20:902-8.
58. Gkrania-Klotsas E, Langenberg C, Sharp SJ, Luben R, Khaw KT, Wareham NJ. Seropositivity and higher immunoglobulin g antibody levels against cytomegalovirus are associated with mortality in the population-based European prospective investigation of cancer-norfolk cohort. Clin Infect Dis. 2013;56:1421-7.
59. Roberts ET, Haan MN, Dowd JB, Aiello AE. Cytomegalovirus antibody levels, inflammation, and mortality among elderly Latinos over 9 years of follow-up. Am J Epidemiol. 2010;172:363-71.
60. Soderberg-Naucler C. Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer? J Intern Med. 2006;259:219-46.
61. Soroceanu L, Cobbs CS. Is HCMV a tumor promoter? Virus Res. 2011;157:193-203.
62. Cinatl J, Scholz M, Kotchetkov R, Vogel JU, Doerr HW. Molecular mechanisms of the modulatory effects of HCMV infection in tumor cell biology. Trends Mol Med. 2004;10:19-23.
63. Michaelis M, Baumgarten P, Mittelbronn M, Driever PH, Doerr HW, Cinatl J, Jr. Oncomodulation by human cytomegalovirus: novel clinical findings open new roads. Med Microbiol Immunol. 2011;200:1-5.
64. Koch S, Larbi A, Ozcelik D, Solana R, Gouttefangeas C, Attig S, et al. Cytomegalovirus infection: a driving force in human T cell immunosenescence. Ann N Y Acad Sci. 2007;1114:23-35.
65. Pawelec G, Akbar A, Beverley P, Caruso C, Derhovanessian E, Fulop T, et al. Immunosenescence and Cytomegalovirus: where do we stand after a decade? Immun Ageing. 2010;7:13.
66. Pacsa AS, Kummerlander L, Pejtsik B, Pali K. Herpesvirus antibodies and antigens in patients with cervical anaplasia and in controls. J Natl Cancer Inst. 1975;55:775-81.
67. Harkins L, Volk AL, Samanta M, Mikolaenko I, Britt WJ, Bland KI, et al. Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. Lancet. 2002;360:1557-63.

68. Harkins LE, Matlaf LA, Soroceanu L, Klemm K, Britt WJ, Wang W, et al. Detection of human cytomegalovirus in normal and neoplastic breast epithelium. Herpesviridae. 2010;1:8.
69. Samanta M, Harkins L, Klemm K, Britt WJ, Cobbs CS. High prevalence of human cytomegalovirus in prostatic intraepithelial neoplasia and prostatic carcinoma. J Urol. 2003;170:998-1002.
70. Martinez-Fierro ML, Leach RJ, Gomez-Guerra LS, Garza-Guajardo R, Johnson-Pais T, Beuten J, et al. Identification of viral infections in the prostate and evaluation of their association with cancer. BMC Cancer. 2010;10:326.
71. Berrington de Gonzalez A, Urban MI, Sitas F, Blackburn N, Hale M, Patel M, et al. Antibodies against six human herpesviruses in relation to seven cancers in black South Africans: a case control study. Infect Agent Cancer. 2006;1:2.
72. Soderberg-Naucler C, Fish KN, Nelson JA. Interferon-gamma and tumor necrosis factor-alpha specifically induce formation of cytomegalovirus-permissive monocyte-derived macrophages that are refractory to the antiviral activity of these cytokines. J Clin Invest. 1997;100:3154-63.
73. Soderberg-Naucler C, Streblow DN, Fish KN, Allan-Yorke J, Smith PP, Nelson JA. Reactivation of latent human cytomegalovirus in CD14(+) monocytes is differentiation dependent. J Virol. 2001;75:7543-54.
74. Stowe RP, Kozlova EV, Yetman DL, Walling DM, Goodwin JS, Glaser R. Chronic herpesvirus reactivation occurs in aging. Exp Gerontol. 2007;42:563-70.
75. Mehta SK, Stowe RP, Feiveson AH, Tyring SK, Pierson DL. Reactivation and shedding of cytomegalovirus in astronauts during spaceflight. J Infect Dis. 2000;182:1761-4.
76. Glaser R, Kiecolt-Glaser JK, Speicher CE, Holliday JE. Stress, loneliness, and changes in herpesvirus latency. J Behav Med. 1985;8:249-60.
77. Stowe RP, Peek MK, Perez NA, Yetman DL, Cutchin MP, Goodwin JS. Herpesvirus reactivation and socioeconomic position: a community-based study. J Epidemiol Community Health. 2010;64:666-71.
78. Sutcliffe S, Till C, Gaydos CA, Jenkins FJ, Goodman PJ, Hoque AM, et al. Prospective study of cytomegalovirus serostatus and prostate cancer risk in the Prostate Cancer Prevention Trial. Cancer Causes Control. 2012;23:1511-8.
79. Huang WY, Hayes R, Pfeiffer R, Viscidi RP, Lee FK, Wang YF, et al. Sexually transmissible infections and prostate cancer risk. Cancer Epidemiol Biomarkers Prev. 2008;17:2374-81.
80. Bergh J, Marklund I, Gustavsson C, Wiklund F, Gronberg H, Allard A, et al. No link between viral findings in the prostate and subsequent cancer development. Br J Cancer. 2007;96:137-9.
81. Richardson AK, Cox B, McCredie MR, Dite GS, Chang JH, Gertig DM, et al. Cytomegalovirus, Epstein-Barr virus and risk of breast cancer before age 40 years: a case-control study. Br J Cancer. 2004;90:2149-52.
82. Cox B, Richardson A, Graham P, Gislefoss RE, Jellum E, Rollag H. Breast cancer, cytomegalovirus and Epstein-Barr virus: a nested case-control study. Br J Cancer. 2010;102:1665-9.

Chapter 2. A prospective study of neutrophil count and the risk of cancer incidence and mortality in the Atherosclerosis Risk in Communities (ARIC) study

Abstract

Several prospective studies have reported a positive association between total white blood cell (WBC) count and cancer incidence and mortality. However, subtypes of total WBC count may have distinct roles in inflammation-mediated tumorigenesis. We examined the relationship between pre-diagnostic neutrophil count, the predominant WBC subtype, and subsequent cancer incidence and mortality in the Atherosclerosis Risk in Communities (ARIC) study. Participants were included if they had a total WBC count within the normal range and no prior cancer at baseline blood draw. Cox proportional hazard models were used to estimate the multivariable-adjusted hazard ratio (HR) and 95% confidence interval (CI) by tertile of neutrophil count. During follow-up, 2,136 incident cancers (1987-2006) and 952 cancer deaths (1987-2008) were detected. Compared to the lowest tertile, the highest neutrophil count tertile was associated with an increased risk of total cancer incidence (HR: 1.11, 95% CI: 1.00, 1.25) and, specifically, lung cancer incidence (HR: 1.59, 95% CI: 1.12, 2.26). In stratified analyses, a significant increased risk was present among current smokers only (HR: 1.30, 95% CI: 1.03, 1.64). The highest tertile of neutrophil count was also associated with an increased risk of cancer mortality, overall (HR: 1.44, 95% CI: 1.22, 1.72), and across strata of sex, race and smoking status. In cancer mortality site-specific analyses, high neutrophil count was associated with an increased risk of lung (HR: 1.66, 95% CI: 1.18, 2.33) and breast cancer mortality (HR: 2.09, 95% CI: 1.10, 3.97). Our findings support a role for low-grade inflammation in tumor development and progression and suggest a specific effect for neutrophils in this process.

Introduction

Inflammation has been implicated in the development and progression of cancer in observational (1-7) and experimental studies (8-11). Total white blood cell (WBC) count is a common clinical marker of non-specific, systemic inflammation. Prospective epidemiological studies have reported a modest association between higher levels of circulating total WBC count, within the clinically normal reference range, and increased risk of both cancer incidence and cancer mortality (2, 6, 7, 12, 13). A similar, significant association was observed even after excluding incident cancers and cancer deaths within the first several years of follow-up to account for possible reverse causality (6, 7, 12, 13). Collectively, these studies support a role for low-grade inflammation in cancer development and progression.

White blood cells (WBC) consist of diverse cell types, which have defined roles in the host immune response (14). These cell types have also been shown to have specific effects on carcinogenesis (8). In particular, neutrophils, the predominant WBC subtype, are activated early in the acute and innate inflammatory responses. These cells are also implicated, more broadly, in the regulation of chronic inflammation and the adaptive immune response (14, 15). Additionally, in vitro and in vivo studies support a direct pro-tumoral role for neutrophils in the tumor microenvironment via the production of reactive oxygen species, cytokines and proteases (15-18). This is consistent with findings in the clinical setting, in which higher levels of circulating neutrophil count at the time of cancer diagnosis were associated with poorer prognosis of renal and lung carcinoma (19-21).

To date, few prospective studies have examined the relationship between WBC subtypes and either cancer incidence or mortality, including only one study evaluating neutrophil count. In this previous study, no association was found between neutrophil count and subsequent cancer incidence in sex- and age-adjusted models. However, factors that may potentially confound or modify this association, such as cigarette smoking and body mass index (BMI), were not assessed (22). To our knowledge, the association between neutrophil count and cancer mortality has not been previously studied.

Furthermore, circulating neutrophil count varies significantly by race, with a lower distribution of counts found in healthy black adults than in whites (23-25). This difference has been linked to a single nucleotide polymorphism (SNP) in the Duffy antigen receptor for chemokines (DARC) (26). It remains unknown whether or not this genetic variant affects the innate immune response, particularly in the context of cancer development and progression.

The present study evaluates the association between neutrophil count, within the clinically normal range, and subsequent cancer incidence and cancer mortality, overall and by race, sex, smoking status, and polymorphisms of the DARC gene. Given the emerging literature on the role of low-grade inflammation in the carcinogenesis pathway, and the specific contribution of neutrophils (8, 10, 11), we hypothesize that higher levels of circulating neutrophils may be associated with an increased risk of cancer incidence and cancer mortality.

Materials and Methods

Study population

This study was conducted in the ARIC study, an ongoing, prospective cohort initiated between 1987 and 1989 to investigate the etiology of atherosclerosis and its sequelae. Men and women (N=15,792), ages 45 to 64 years, were enrolled from four U.S. communities, Forsyth County, NC; Jackson, MS; Washington County, MD; and suburban Minneapolis, MN. Participants were identified using probability sampling in Minneapolis and Washington County, while in Jackson, blacks were exclusively recruited and in Forsyth County blacks were oversampled (27). Participants from Washington County were not included in these analyses as baseline WBC subtype count was missing for more than 85% of participants (28). At enrollment, participants provided a blood sample and reported information on sociodemographic factors, medical history, reproductive history, physical activity, alcohol and tobacco use and other lifestyle behaviors via standardized questionnaires.

The analytic cohort included both men and women that met the following eligibility criteria: (1) not residing in Washington County, MD (N=11,792); (2) no personal history of cancer, excluding cases of non-melanoma skin cancer (N=11,175); (3) white or black race (N=11,140); (4) total WBC count within two standard deviations of the mean in whites and blacks, separately (range: 3.0 to 10.9 x 10^9 cells/L) (N=10,351); (5) not missing baseline neutrophil count (N=10,348); and (6) not missing information on cancer incidence (N=10,189) or mortality (N=10,313).

Exposure ascertainment

Total and differential WBC counts were measured at baseline and three years later at Visit 2. In the main analyses, baseline WBC subtype counts were evaluated. Following venipuncture, samples were stored at 4°C and within 24 hours total WBC count was measured using automated particle counters in local, independent clinical laboratories. The WBC subtypes were measured as a proportion of total WBCs and counts were subsequently calculated by multiplying this proportion by total WBC count. Based on repeat testing of individuals conducted one to two weeks apart, reliability coefficients for total WBC count were estimated to be greater than 0.96 for each laboratory (29, 30).

Outcome ascertainment

The incidence of a first primary cancer, including date of diagnosis and site of cancer, was ascertained from study initiation through December 31, 2006 (1, 30). Cancer incidence was primarily identified by linkage to well-established state and/or county cancer registries that have a high completeness (≥90%) of cancer data (30). Hospital surveillance was used to identify cancer cases in Jackson prior to establishment of the Mississippi state cancer registry in 1996 (31), as well as all additional cancer cases for the other study sites (30, 32). At present, data on stage at diagnosis, cancer subtype and treatment are not consistently available for all cancers and, therefore, were not incorporated in analyses.

Vital status was available through December 31, 2008. Deaths were identified through contact with relatives, physician or designated contact, or through a search of obituaries, funeral and hospital records, death certificates and the National Death Index (NDI). The date and cause of death were confirmed by death certificate for all reported deaths. Cause of death was coded using the Ninth International Statistical Classification of Diseases and Related Health Problems (ICD-9) for deaths through 1998 and ICD-10 for all subsequent deaths. Cause-specific mortality from death certificate was available for 98% of decedents (33).

Assessment of covariates

Participants reported their highest education attainment, regular alcohol use, and intake of aspirin in the two weeks prior to baseline study visit. They also provided information on current cigarette smoking status and, if applicable, the average number of years of smoking and cigarettes smoked per day. These values were used to calculate pack-years ([cigarettes per day*years smoking]/20 cigarettes per pack) among ever smokers. Exposure to environmental tobacco smoke (ETS) was defined as being in close proximity to smokers for more than 1 hour per week (34, 35). Body mass index (BMI), calculated as weight divided by height squared [weight (kg)/height(m)^2], and waist circumference were collected by trained technicians at baseline study visit. A history of cardiovascular disease (CVD) was defined as having a prior diagnosis of angina pectoris, coronary heart disease, intermittent claudication or stroke. Participants were categorized as having hypertension if they reported use of any hypertensive medications or if they had systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Diabetes was defined

as having a fasting glucose level ≥126 mg/dl, a non-fasting glucose level ≥200 mg/dl, a self-reported physician diagnosis of diabetes, or use of blood sugar lowering medications in the two weeks prior to enrollment. Women were categorized as being premenopausal if they had a menstrual cycle within two years of baseline or postmenopausal (36). Women missing information on menopausal status were categorized as being postmenopausal if they were 55 years of age or older. Postmenopausal hormone use was categorized as current, former or never (36).

Genotyping of the rs2814778 polymorphism in the *DARC* gene was performed using the ABI TaqMan technology, and the proportion of European ancestry was measured using a genome-wide admixture mapping scan with 1,350 ancestry-informative SNPs. Genotyping methods have been described previously (26, 37).

Statistical analyses

Baseline neutrophil count was categorized as tertiles based on race-specific cut points, given the significant differences in the mean and standard deviation in whites and blacks (25, 38). Race-specific tertiles of neutrophil count (whites tertile 1: 1.100-3.135, tertile 2: 3.136-4.189, tertile 3: 4.200-9.180; blacks tertile 1: 0.333-2.035, tertile 2: 2.040, 3.024, tertile 3: 3.024, 7.600 $x10^9$ cells/L) were then combined across the total analytic study population. Baseline descriptive characteristics were compared by neutrophil count tertile using the chi-square test for categorical variables or ANOVA for continuous variables.

Cox proportional hazards models were used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for cancer incidence and cancer mortality by tertile of neutrophil count. Tests for linear trend across categories of neutrophil count were calculated by introducing an ordinal variable into models, which represented the rank order of the categories. In analyses of cancer incidence, follow-up time was accrued from age at baseline blood draw, with the origin defined as age 40 years and staggered late entries for persons over age 40 at baseline, to the first of the following events: (1) age at first primary cancer diagnosis, (2) age at death, or (3) age at end of follow-up (December 31, 2006). In analyses of cancer mortality, individuals were followed from age at baseline blood draw to (1) age at death or (2) age at end of follow-up (December 31, 2008). In models in which time accrued from year at baseline blood draw to (1) year at first primary cancer diagnosis or cancer death, (2) year at other death, or (3) year at end of follow-up, similar HRs were estimated and are not presented here. Multivariable models included the baseline covariates sex, race (white, black), study site (Jackson, MS, Forsyth County, Minneapolis, MN), BMI (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, $\geq$35.0 kg/m^2), waist circumference (continuous), education attainment (<high school diploma, high school diploma, >high school or college graduate, graduate school), cigarette smoking status (never, former, current), pack-years (continuous), ETS ($\leq$1 hour/week, >1 hour/week), alcohol intake (g/wk), aspirin use in two weeks prior to blood draw (yes/no), and medical history (yes/no) of CVD, hypertension, and diabetes. Missing pack-year and waist circumference information was replaced with the median value. Further adjustment by counts of the other WBC subtypes (lymphocyte count tertile ($\leq$1.65, 1.66-2.14, $\geq$2.15 x10^9 cells/L), monocyte count tertile ($\leq$0.272, 0.273-0.399, $\geq$0.400 x10^9 cells/L),

eosinophil count tertile ($\leq$0.07, 0.07-0.174, $\geq$0.175 x10^9 cells/L) and basophil category (<0.03, $\geq$0.03 x10^9 cells/L)) was also performed. Effect modification between neutrophil count and covariates known to modulate immune function, including race, sex, age, cigarette smoking status, aspirin use and BMI, was assessed by introducing a cross-product term in models and using the Wald test to test for statistical significance. Additionally, among black participants, effect modification by the presence of the Duffy null polymorphism and percent European ancestry was evaluated. The proportional hazards assumption was assessed by introducing an interaction term between neutrophil count and follow-up time into models. In all cases, the interaction term was not statistically significant, confirming this assumption. Rates of cancer incidence and cancer mortality were standardized to the age, race and sex distribution of the analytic cohort.

Cancer site-specific analyses were conducted in models of cancer incidence and cancer mortality for the four most common cancers (i.e., female breast cancer, colorectal cancer, lung cancer, and prostate cancer, Appendix A, Table A.1). Additionally, the following sensitivity analyses were performed: (1) excluding incident cancer cases within one and five years from baseline blood draw in order to address the possibility of bias due to reverse causation; (2) excluding persons who used aspirin in the two weeks prior to baseline; (3) excluding participants with neutrophil counts greater than two standard deviations from the mean, in whites and blacks separately, to more rigorously restrict analyses to persons without acute inflammation or underlying inflammatory disease; and (4) using neutrophil count measured at Visit 2 as the exposure of interest and, accordingly, accumulating time at risk from age at Visit 2 (1990-1992). Lastly, in cancer

mortality analyses, sub-distribution hazard ratios were estimated using the Fine and Gray approach to account for the issue of competing risks (39). All analyses were conducted using STATA version 11.2 (Stata Corporation, College Station, TX, 2012).

Results

The distributions of neutrophil count by select baseline characteristics are shown in Table 2.1. The median neutrophil count was 3.60 x 10^9 cells/L (interquartile range (IQR): 2.91, zhighest neutrophil count tertile were significantly more likely to be male, less educated, have a higher BMI and waist circumference, consume more alcohol, and have a medical history of CVD, hypertension and diabetes, compared to those in the lowest tertile. Additionally, exposure to tobacco smoke, based on current cigarette smoking status, pack-years and ETS, was greater in the highest tertile of neutrophil count compared to the lowest. In whites, there were similar differences in the distribution of baseline characteristics by neutrophil count tertile, while, in blacks, there was no significant differences in sex, education attainment, BMI, pack-years, exposure to ETS or hypertension (Appendix A, Table A.2).

Cancer incidence

Between 1987 and 2006, 2,136 incident cancers developed over a median follow-up of 18.1 years. Table 2.2 presents the multivariable-adjusted associations between neutrophil count tertile and cancer incidence overall and stratified by select factors. Overall, the highest neutrophil count tertile was associated with a 1.11-fold (95% CI: 1.00, 1.25, p-trend=0.06) increased risk of cancer incidence, compared to the lowest tertile. There was

no significant interaction between neutrophil count and race, sex, cigarette smoking status, BMI or aspirin use (all p-interaction terms $\geq$0.1). However, in stratified analyses, neutrophil count was significantly associated with cancer incidence among current smokers (HR: 1.30, 95% CI: 1.03, 1.64, p-trend=0.05), but not among former or never smokers. The significant association between high neutrophil count and cancer incidence in the total study population persisted after excluding incident cancer events within one and five years of follow up and among persons who did not use aspirin in the two weeks prior to blood draw. Similar significant results were also found in analyses excluding persons with neutorphil counts greater than two standard deviations from the mean. In analyses using neutrophil count measured at Visit 2, the association between the highest tertile of neutrophil count and cancer incidence was no longer statistically significant (HR: 1.08, 95% CI: 0.94, 1.25).

In cancer-site specific analyses, neutrophil count tertile was associated with an increased risk of developing lung cancer (HR: 1.59, 95% CI: 1.12, 2.26, p-trend=0.01) and a non-significant increased risk of colorectal cancer incidence (HR: 1.35, 95% CI: 0.95, 1.92, p-trend=0.10). There was no association with breast cancer incidence in females or prostate cancer incidence in males (Figure 2.1).

Cancer mortality

Between 1987 and 2008, 952 cancer deaths occurred during a median follow-up of 21.7 years. In the total study population, the highest neutrophil count tertile was associated with a 44% (HR: 1.44, 95% CI: 1.22, 1.72, p-trend<0.0001) increased risk of cancer

mortality, compared to the lowest tertile of neutrophil count (Table 2.3). This association remained significant after excluding incident cancer cases within one and five years from baseline and in analyses restricted to participants with neutrophil counts within two standard deviations of the mean. There was no evidence of a statistical interaction by race, sex, cigarette smoking status, BMI or aspirin use (all p-interaction terms $\geq$0.1), and a significant positive association between neutrophil count tertile and cancer mortality held across strata of cigarette smoking status (current smokers HR: 1.37, 95% CI: 1.02, 1.84, p-trend=0.02; former smokers HR: 1.42, 95% CI: 1.07, 1.89, p-trend=0.02; never smokers HR: 1.47, 95% CI: 1.06, 2.05, p-trend=0.02) (Table 3). In analyses using neutrophil count measured at Visit 2, a significant positive association persisted between the highest tertile of neutrophil count and total cancer mortality (HR: 1.39, 95% CI: 1.11, 1.76).

In cancer-site specific analyses, we observed an increased risk of lung cancer death (HR: 1.66, 95% CI: 1.18, 2.33, p-trend=0.005) and female breast cancer death (HR: 2.09, 95% CI: 1.10, 3.97, p-trend=0.02) when comparing the highest neutrophil count tertile to the lowest (Figure 2.2). Upon excluding deaths due to lung cancer, a significant positive association remained between the highest tertile of neutrophil count and non-lung cancer mortality in never smokers (Appendix A, Table A.3). Furthermore, in analyses restricted to postmenopausal women, a positive, but non-significant association was observed for breast cancer deaths (HR: 2.25, 95% CI: 0.96, 5.27). There was no association between neutrophil count and colorectal or prostate cancer mortality (Figure 2.2).

Additionally, the highest tertile of neutrophil count was associated with an increased risk of CVD death (HR: 1.45, 95% CI: 1.23, 1.71, p-trend<0.0001) and non-cancer mortality (HR: 1.62, 95% CI: 1.40, 1.87, p-trend<0.0001), compared to the lowest tertile. In competing risk analyses, accounting for deaths from causes other than cancer, the association between the highest neutrophil count tertile and cancer mortality persisted (sub-distribution HR: 1.38, 95% CI: 1.16, 1.64).

Other WBC subtypes

Correlations across baseline WBC subtype counts are presented in Appendix A, Table A.4. In models of cancer incidence and mortality, mutual adjustment for the other WBC subtypes did not significantly alter risk estimates (Table 2.4). Additionally, for comparison with our main models, we also estimated the risk of cancer incidence and cancer mortality by quartile of total WBC count (Appendix A, Tables A.5). In multivariable-adjusted models, there was no association between total WBC count and cancer incidence, while the highest quartile of total WBC count was associated with a 47% (HR: 1.47, 95% CI: 1.20, 1.81) increased risk of cancer mortality.

Stratification by genetic markers

In a subset of black participants with available genetic data (N=3,131), the median proportion of European ancestry was 0.17 (IQR: 0.11, 0.22) and 70% were carriers of the Duffy null polymorphism. The highest tertile of neutrophil count was more strongly associated with both cancer incidence (HR: 1.27, 95% CI: 0.96, 1.68) and cancer mortality (HR: 1.40, 95% CI: 0.95, 2.07) in carriers of the Duffy null polymorphism than

in non-carriers ($HR_{cancer\ incidence}$: 0.94, 95% CI: 0.51, 1.76 and $HR_{cancer\ mortality}$: 1.01, 95% CI: 0.42, 2.42), although this difference was not statistically significant (p-interaction=0.58 and 0.72, respectively).

Additionally, percent European ancestry significantly modified the association between neutrophil count tertile and cancer incidence (p-interaction=0.009), such that higher levels of neutrophil count was more strongly associated with cancer incidence in blacks with lower percent European ancestry. However, there was no significant interaction in models of cancer mortality.

Discussion

In this large, prospective cohort study, high pre-diagnostic neutrophil count was associated with an increased risk of total cancer incidence, independent of the effects of the other WBC subtypes. However, in further analyses, this association was limited to current smokers and was specific to cases of lung cancer. High neutrophil count was also associated with an increased risk of total cancer mortality. This significant association persisted after mutual adjustment for the other WBC subtypes and after excluding cancer cases within the first five years of follow-up to account for the presence of subclinical disease. Additionally, a significant positive association held across strata of sex, race, and cigarette smoking status, In cancer mortality site-specific analyses, significant associations were observed for lung and breast cancers.

Although neutrophils are primarily involved in the acute inflammatory response, these cells also have a role in regulating chronic inflammation. Thus, neutrophils, may be an informative marker of inflammation. There is also evidence from in vitro and in vivo studies to suggest that neutrophils may directly exert pro-tumoral effects in the tumor microenvironment (16, 40, 41), generally, and in breast and lung cancers, specifically (20, 42, 43). Our study expands upon these findings by reporting, for the first time, that higher neutrophil count, measured years prior to the onset of cancer, may also have pro-tumoral effects, particularly with respect to tumor progression.

Only one previous study has examined the association between circulating neutrophil count and subsequent cancer incidence. In that study, conducted among 669 Danish adults, no association was found in age- and sex-adjusted models (22). In contrast, in the present study, we found a positive association between neutrophil count and cancer incidence in multivariable-adjusted models. However, given that this association was limited to current cigarette smokers, our finding may be explained by residual confounding by tobacco use, despite having adjusted for pack-years and ETS. There is little data to support the alternate explanation, that tobacco may modify the function of neutrophil count (44, 45).

To our knowledge, this is the first prospective study to examine the association between pre-diagnostic neutrophil count and cancer mortality. Our finding of a significant positive association, regardless of cigarette smoking status, is consistent with previous studies, in which total WBC count was positively associated with total (6, 7, 12), lung and breast

cancer mortality (7). The stronger association with cancer mortality than with cancer incidence in this study suggests that the neutrophil-mediated inflammatory response may lead to the development of more aggressive disease. Alternatively, higher baseline neutrophil count or its sequelae may impact treatment resistance and, in turn, prognosis. In the present study, we were unable to evaluate these possibilities, as information on stage at diagnosis and treatment were not consistently available.

Although there was no evidence of effect modification by self-reported race in the present study, we did observe a significantly stronger association between neutrophil count and cancer incidence in individuals with lower percent European ancestry, suggesting that a genetic variant(s) related to race may affect neutrophil function, particularly in the context of cancer initiation. One possible candidate is the Duffy null polymorphism, which is present nearly exclusively in black populations and has been linked to lower circulating neutrophil count (26). While we did find a stronger association between neutrophil count and cancer incidence and mortality in carriers of the Duffy null polymorphism, this interaction did not reach statistical significance. This is not entirely unexpected as any negative health effects associated with this genetic variant are expected to be subtle given the high frequency of this polymorphism in black populations (26).

Certain limitations of this study warrant further consideration. First, we utilized a one-time measurement of neutrophil count. However in another study with over 40 years of follow-up, accounting for time-varying measures of total WBC count, measured every

two years, as compared to using a single baseline measurement, did not appreciably alter risk estimates (46). Additionally, in a subset of the analytic cohort, the correlation between neutrophil counts measured at baseline and Visit 2, three years later, was 0.65, indicating that this marker is fairly stable in the short-term, and in analyses using neutrophil count measured at Visit 2, similar associations were found for both cancer incidence and mortality. Another limitation of this study is that the effects of race cannot be completely differentiated from geography as nearly all blacks were recruited from Jackson, MS while whites were identified at the other three study sites.

This study also has several strengths. The ARIC study is a prospective, population-based cohort with over 20 years of follow-up for cancer outcomes and good representation of both whites and blacks. Few other cohorts with prospectively collected information on cancer endpoints have also measured baseline WBC subtype counts. Additionally, detailed information was available on a wide-array of socio-demographic characteristics, lifestyle risk factors and medical history, as well as genetic markers.

Our findings provide the first prospective evidence linking the neutrophil-mediated inflammatory response in healthy men and women to subsequent cancer incidence and mortality. This adds to the mounting evidence linking low-grade inflammation to tumor development and progression and expands upon laboratory data suggesting a specific role for neutrophils in carcinogenesis. Future studies are necessary to explore the role of the downstream products of neutrophil activation and other WBC subtypes in cancer development and progression.

Acknowledgments

The authors thank the staff and participants of the ARIC study for their important contributions and the ARIC Cancer Working Group for their feedback on this work.

References

1. Prizment AE, Anderson KE, Visvanathan K, Folsom AR. Association of inflammatory markers with colorectal cancer incidence in the atherosclerosis risk in communities study. Cancer Epidemiol Biomarkers Prev. 2011;20:297-307.
2. Godsland IF, North BV, Johnston DG. Simple indices of inflammation as predictors of death from cancer or cardiovascular disease in a prospective cohort after two decades of follow-up. QJM. 2010.
3. Heikkila K, Harris R, Lowe G, Rumley A, Yarnell J, Gallacher J, et al. Associations of circulating C-reactive protein and interleukin-6 with cancer risk: findings from two prospective cohorts and a meta-analysis. Cancer Causes Control. 2009;20:15-26.
4. Il'yasova D, Colbert LH, Harris TB, Newman AB, Bauer DC, Satterfield S, et al. Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. Cancer Epidemiol Biomarkers Prev. 2005;14:2413-8.
5. Chaturvedi AK, Caporaso NE, Katki HA, Wong HL, Chatterjee N, Pine SR, et al. C-reactive protein and risk of lung cancer. J Clin Oncol. 2010;28:2719-26.
6. Erlinger TP, Muntner P, Helzlsouer KJ. WBC count and the risk of cancer mortality in a national sample of U.S. adults: results from the Second National Health and Nutrition Examination Survey mortality study. Cancer Epidemiol Biomarkers Prev. 2004;13:1052-6.
7. Margolis KL, Rodabough RJ, Thomson CA, Lopez AM, McTiernan A. Prospective study of leukocyte count as a predictor of incident breast, colorectal, endometrial, and lung cancer and mortality in postmenopausal women. Arch Intern Med. 2007;167:1837-44.
8. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140:883-99.
9. Kundu JK, Surh YJ. Inflammation: gearing the journey to cancer. Mutat Res. 2008;659:15-30.
10. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer. 2006;6:24-37.
11. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420:860-7.
12. Shankar A, Wang JJ, Rochtchina E, Yu MC, Kefford R, Mitchell P. Association between circulating white blood cell count and cancer mortality: a population-based cohort study. Arch Intern Med. 2006;166:188-94.
13. Van Hemelrijck M, Holmberg L, Garmo H, Hammar N, Walldius G, Binda E, et al. Association between Levels of C-Reactive Protein and Leukocytes and Cancer: Three Repeated Measurements in the Swedish AMORIS Study. Cancer Epidemiol Biomarkers Prev. 2011;20:428-37.
14. Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J. Harrison's Principles of Internal Medicine. 18 ed: McGraw-Hill; 2011.
15. Brandau S, Dumitru CA, Lang S. Protumor and antitumor functions of neutrophil granulocytes. Semin Immunopathol. 2013;35:163-76.
16. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. Cancer Cell. 2009;16:183-94.

17. Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol. 2011;11:519-31.
18. Jablonska J, Leschner S, Westphal K, Lienenklaus S, Weiss S. Neutrophils responsive to endogenous IFN-beta regulate tumor angiogenesis and growth in a mouse tumor model. J Clin Invest. 2010;120:1151-64.
19. Jensen HK, Donskov F, Marcussen N, Nordsmark M, Lundbeck F, von der Maase H. Presence of intratumoral neutrophils is an independent prognostic factor in localized renal cell carcinoma. J Clin Oncol. 2009;27:4709-17.
20. Wislez M, Rabbe N, Marchal J, Milleron B, Crestani B, Mayaud C, et al. Hepatocyte growth factor production by neutrophils infiltrating bronchioloalveolar subtype pulmonary adenocarcinoma: role in tumor progression and death. Cancer Res. 2003;63:1405-12.
21. Donskov F, von der Maase H. Impact of immune parameters on long-term survival in metastatic renal cell carcinoma. J Clin Oncol. 2006;24:1997-2005.
22. Sajadieh A, Mouridsen MR, Selmer C, Intzilakis T, Nielsen OW, Haugaard SB. Monocyte number associated with incident cancer and mortality in middle-aged and elderly community-dwelling Danes. Eur J Cancer. 2011;47:2015-22.
23. Haddy TB, Rana SR, Castro O. Benign ethnic neutropenia: what is a normal absolute neutrophil count? J Lab Clin Med. 1999;133:15-22.
24. Freedman DS, Gates L, Flanders WD, Van Assendelft OW, Barboriak JJ, Joesoef MR, et al. Black/white differences in leukocyte subpopulations in men. Int J Epidemiol. 1997;26:757-64.
25. Lim EM, Cembrowski G, Cembrowski M, Clarke G. Race-specific WBC and neutrophil count reference intervals. Int J Lab Hematol. 2010;32:590-7.
26. Reich D, Nalls MA, Kao WH, Akylbekova EL, Tandon A, Patterson N, et al. Reduced neutrophil count in people of African descent is due to a regulatory variant in the Duffy antigen receptor for chemokines gene. PLoS Genet. 2009;5:e1000360.
27. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. Am J Epidemiol. 1989;129:687-702.
28. Prizment AE, Anderson KE, Visvanathan K, Folsom AR. Inverse association of eosinophil count with colorectal cancer incidence: atherosclerosis risk in communities study. Cancer Epidemiol Biomarkers Prev. 2011;20:1861-4.
29. Nieto FJ, Szklo M, Folsom AR, Rock R, Mercuri M. Leukocyte count correlates in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol. 1992;136:525-37.
30. Ahmed RL, Schmitz KH, Anderson KE, Rosamond WD, Folsom AR. The metabolic syndrome and risk of incident colorectal cancer. Cancer. 2006;107:28-36.
31. Mississippi Cancer Registry Reporting Manual Revised 2011. Manual2011.
32. Prizment AE, Folsom AR, Dreyfus J, Anderson KE, Visvanathan K, Joshu CE, et al. Plasma C-reactive protein, genetic risk score, and risk of common cancers in the Atherosclerosis Risk in Communities study. Cancer Causes Control. 2013;24:2077-87.
33. Rose KM, Eigenbrodt ML, Biga RL, Couper DJ, Light KC, Sharrett AR, et al. Orthostatic hypotension predicts mortality in middle-aged adults: the Atherosclerosis Risk In Communities (ARIC) Study. Circulation. 2006;114:630-6.

34. Howard G, Wagenknecht LE, Burke GL, Diez-Roux A, Evans GW, McGovern P, et al. Cigarette smoking and progression of atherosclerosis: The Atherosclerosis Risk in Communities (ARIC) Study. JAMA. 1998;279:119-24.
35. Kan H, Heiss G, Rose KM, Whitsel EA, Lurmann F, London SJ. Prospective analysis of traffic exposure as a risk factor for incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study. Environ Health Perspect. 2008;116:1463-8.
36. Nabulsi AA, Folsom AR, Szklo M, White A, Higgins M, Heiss G. No association of menopause and hormone replacement therapy with carotid artery intima-media thickness. Atherosclerosis Risk in Communities (ARIC) Study Investigators. Circulation. 1996;94:1857-63.
37. Cheng CY, Reich D, Coresh J, Boerwinkle E, Patterson N, Li M, et al. Admixture mapping of obesity-related traits in African Americans: the Atherosclerosis Risk in Communities (ARIC) Study. Obesity (Silver Spring). 2010;18:563-72.
38. Tian N, Penman AD, Mawson AR, Manning RD, Jr., Flessner MF. Association between circulating specific leukocyte types and blood pressure: the atherosclerosis risk in communities (ARIC) study. J Am Soc Hypertens. 2010;4:272-83.
39. Fine J, Gray R. A proportional Hazards Model for the Subdistribution of Competing Risk. Journal of the American Statistical Association. 1999;94:496-509.
40. Mantovani A, Savino B, Locati M, Zammataro L, Allavena P, Bonecchi R. The chemokine system in cancer biology and therapy. Cytokine Growth Factor Rev. 2010;21:27-39.
41. Nozawa H, Chiu C, Hanahan D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. Proc Natl Acad Sci U S A. 2006;103:12493-8.
42. Queen MM, Ryan RE, Holzer RG, Keller-Peck CR, Jorcyk CL. Breast cancer cells stimulate neutrophils to produce oncostatin M: potential implications for tumor progression. Cancer Res. 2005;65:8896-904.
43. Jorcyk CL, Holzer RG, Ryan RE. Oncostatin M induces cell detachment and enhances the metastatic capacity of T-47D human breast carcinoma cells. Cytokine. 2006;33:323-36.
44. Sorensen LT, Nielsen HB, Kharazmi A, Gottrup F. Effect of smoking and abstention on oxidative burst and reactivity of neutrophils and monocytes. Surgery. 2004;136:1047-53.
45. Guo X, Wang WP, Ko JK, Cho CH. Involvement of neutrophils and free radicals in the potentiating effects of passive cigarette smoking on inflammatory bowel disease in rats. Gastroenterology. 1999;117:884-92.
46. Ruggiero C, Metter EJ, Cherubini A, Maggio M, Sen R, Najjar SS, et al. White blood cell count and mortality in the Baltimore Longitudinal Study of Aging. J Am Coll Cardiol. 2007;49:1841-50.

Table 2.1 Baseline characteristics by tertile of neutrophil count in ARIC participants, 1987-1989

	Neutrophil Count[a]		
	Tertile 1	**Tertile 2**	**Tertile 3**
Total, N	3,463	3,432	3,442
Study Site[b], %			
Jackson, MS	33.7	30.9	30.0
Forsyth County, NC	31.1	34.9	35.5
Minneapolis, MN	35.3	34.2	34.5
Male, %	41.1	47.1	47.2
Blacks, %	35.9	36.0	36.0
Age (years), Mean (SD)	53.8 (5.6)	54.0 (5.8)	53.9 (5.9)
Education, %			
<High school	20.6	22.2	23.1
High school or college graduate	37.5	39.2	40.2
Graduate school	41.9	38.6	36.7
Missing, N	4	8	5
BMI (kg/m^2), %			
<18.5	0.6	0.6	1.2
18.5-24.99	36.1	30.5	28.8
25.0 29.99	39.5	40.1	39.5
30.0-34.99	17.2	19.3	19.2
≥35.00	6.7	9.5	11.1
Missing, N	2	2	5
Waist Circumference (cm), Mean (SD)	94.5 (13.2)	97.2 (13.7)	98.2 (14.5)
Missing, N	0	0	3
Cigarette Smoking Status, %			
Never	51.5	41.4	29.8
Former	34.9	34.7	27.9
Current	13.6	23.9	42.3
Missing, N	3	3	2
Pack-Years[c], Mean (SD)	23.5 (21.8)	24.3 (21.7)	25.5 (21.9)
Missing, N	60	66	64
ETS[d] (hours/week), %			
≤1	35.5	30.3	23.4
>1	64.5	69.7	76.6
Missing, N	25	19	18
Alcohol Intake[e], Mean (SD) (g/wk)	96.5 (101.0)	109.4 (131.5)	124.8 (151.7)
Missing, N	17	34	17
CVD[f], %	7.5	9.6	12.2
Hypertension[g], %	32.2	36.4	37.0
Missing, N	0	2	1

Table 2.1 (continued) Baseline characteristics by tertile[a] of neutrophil count in ARIC participants

	Neutrophil Count		
	Tertile 1	**Tertile 2**	**Tertile 3**
Diabetes[h], %	7.8	11.5	16.1
Missing, N	3	4	7
Aspirin used[i], %	44.0	44.8	46.3
Missing, N	29	32	21

Abbreviations: BMI, body mass index; SD, standard deviation; ETS, environmental tobacco smoke; CVD, cardiovascular disease. [a] Neutrophil count tertiles defined based on race-specific cut-offs and combined across the analytic cohort. [b]Participants from Washington County, MD were excluded from analyses due to substantial missing WBC subtype count data at this site. [c]Pack-years among ever smokers only. Pack-years was calculated as the average number of cigarettes smoked per day multiplied by the number of years of smoking and divided by 20. [d]ETS defined as the average number of hours per week of close contact with people when they are smoking. [e]Alcohol intake among participants who reported usually having at least one drink per week. [f]CVD defined as having a history of angina pectoris, coronary heart disease, intermittent claudication, or stroke. [g]Hypertension defined as use of any hypertensive medications, systolic blood pressure $\geq$140 mmHg, or diastolic blood pressure $\geq$90 mmHg. [h]Diabetes defined as having a fasting glucose level $\geq$126 mg/dl, non-fasting glucose level $\geq$200 mg/dl, a physician diagnosis of diabetes, or using sugar-lowering medications in two weeks prior to enrollment. [i]Aspirin use in two weeks prior to study enrollment.

Table 2.2 Multivariable-adjusted hazard ratios for cancer incidence by tertile of neutrophil count, stratified by race, sex and cigarette smoking status, in the ARIC study, 1987-2006

Neutrophil Count by Tertile[a]	N	Person-Years	Age-Standardized Rate[b]	HR (95% CI) [c]
Overall				
1	660	56777.3	193.8	1.00 (Reference)
2	700	54285.1	208.1	1.04 (0.93, 1.16)
3	766	51924.3	222.8	1.11 (1.00, 1.25)
P-Trend				0.06
Whites				
1	435	36907.5	198.3	1.00 (Reference)
2	462	35535.8	209.9	1.00 (0.87, 1.14)
3	537	34126.6	243.8	1.11 (0.97, 1.28)
P-Trend				0.12
Blacks				
1	225	19869.8	185.8	1.00 (Reference)
2	247	18749.3	204.5	1.15 (0.96, 1.38)
3	229	17797.7	185.6	1.10 (0.91, 1.34)
P-Trend				0.31
Men				
1	327	22633.5	236.7	1.00 (Reference)
2	388	24948.7	241.5	1.03 (0.89, 1.20)
3	438	2360.3	263.4	1.15 (0.99, 1.34)
P-Trend				0.07
Women[d]				
1	333	34143.9	163.3	1.00 (Reference)
2	321	29336.5	177.7	1.07 (0.91, 1.24)
3	328	28294.0	186.0	1.08 (0.92, 1.27)
P-Trend				0.36
Current Smokers				
1	97	7469.3	213.4	1.00 (Reference)
2	205	12051.5	254.9	1.27 (0.99, 1.63)
3	373	21347.6	259.1	1.30 (1.03, 1.64)
P-Trend				0.05
Former Smokers				
1	252	19604.4	219.4	1.00 (Reference)
2	244	19013.7	204.4	0.92 (0.77, 1.10)
3	216	14693.1	218.2	1.04 (0.86, 1.25)
P-Trend				0.76
Never Smokers				
1	311	29665.3	176.2	1.00 (Reference)
2	259	23196.9	184.6	1.06 (0.89, 1.25)
3	177	15883.6	172.5	1.07 (0.89, 1.30)
P-Trend				0.44

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Neutrophil count tertiles defined based on race-specific cut-offs and combined across the analytic cohort. [b]Incidence rate per 1,000 individuals from 1987 to 2006 standardized to the age, race and sex distribution of the analytic cohort. [c]Models adjusted for study

Table 2.2 (continued) Multivariable-adjusted hazard ratios for cancer incidence by tertile of neutrophil count, stratified by race, sex and cigarette smoking status, in the ARIC study, 1987-2006

site (Jackson, Forsyth County, Minneapolis), race (white, black), sex, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (≤1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [d]Additionally adjusted for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user).

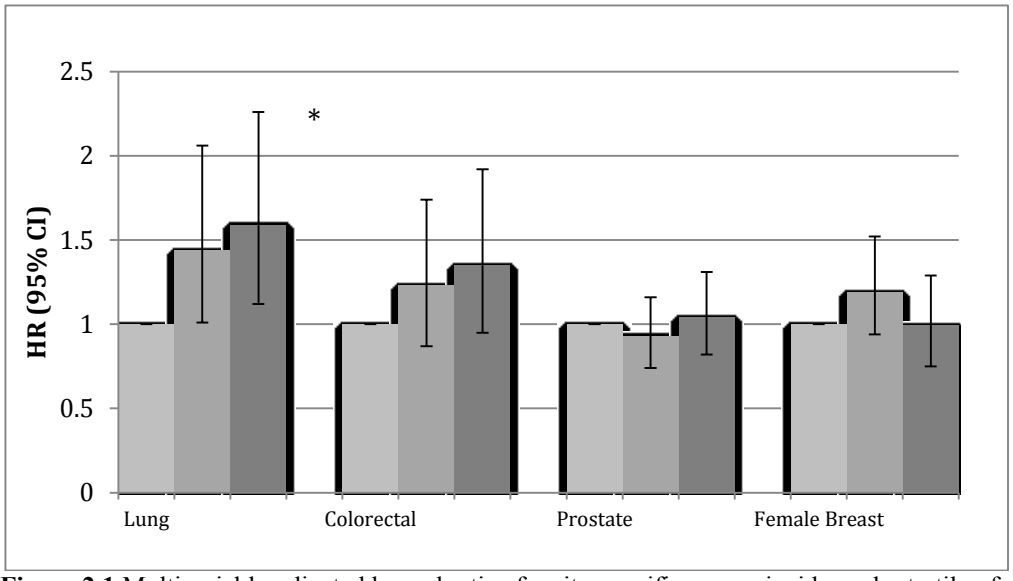

Figure 2.1 Multivariable-adjusted hazard ratios for site-specific cancer incidence by tertile of neutrophil count in the ARIC study, 1987-2006. Light gray=tertile 1; Medium gray=tertile 2; Dark gray=tertile 3. Neutrophil count tertiles defined based on race-specific cut-offs and combined across the analytic cohort. Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), sex, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke ($\leq$1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. Models of female breast cancer were additionally adjusted for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user). Asterisk indicates p-value<0.05.

Table 2.3 Multivariable-adjusted hazard ratios for cancer mortality by tertile of neutrophil count, stratified by race, sex and cigarette smoking status, in the ARIC study, 1987-2008

Neutrophil Count by Tertile[a]	N	Person-Years	Age-Standardized Rate[b]	HR (95% CI)[c]
Overall				
1	236	70232.2	69.7	1.00 (Reference)
2	297	67064.1	87.3	1.13 (0.95, 1.35)
3	418	63627.6	121.5	1.44 (1.22, 1.72)
P-Trend				<0.0001
Whites				
1	139	46230.9	64.4	1.00 (Reference)
2	178	44179.8	80.8	1.07 (0.86, 1.35)
3	284	42013.4	129.2	1.47 (1.18, 1.84)
P-Trend				<0.0001
Blacks				
1	97	24001.3	79.2	1.00 (Reference)
2	119	22884.3	98.9	1.22 (0.93, 1.60)
3	134	21614.2	107.7	1.37 (1.04, 1.79)
P-Trend				0.03
Men				
1	127	28080.2	95.1	1.00 (Reference)
2	175	30647.7	109.8	1.10 (0.87, 1.39)
3	237	29132.9	141.7	1.28 (1.02, 1.61)
P-Trend				0.03
Women[d]				
1	109	42152.1	53.1	1.00 (Reference)
2	122	36416.5	67.6	1.15 (0.88, 1.49)
3	181	34494.6	103.1	1.63 (1.26, 2.09)
P-Trend				<0.0001
Current Smokers				
1	60	8806.4	120.4	1.00 (Reference)
2	118	14760.7	145.8	1.15 (0.83, 1.57)
3	236	25868.4	160.9	1.37 (1.02, 1.84)
P-Trend				0.02
Former Smokers				
1	95	24383.1	87.9	1.00 (Reference)
2	97	23468.1	80.9	0.98 (0.73, 1.30)
3	110	18021.5	112.0	1.42 (1.07, 1.89)
P-Trend				0.02
Never Smokers				
1	81	36998.5	45.4	1.00 (Reference)
2	81	28796.3	58.5	1.22 (0.89, 1.66)
3	72	19737.7	67.0	1.47 (1.06, 2.05)
P-Trend				0.02

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Neutrophil count tertiles defined based on race-specific cut-offs and combined across the analytic cohort. [b]Incidence rate per 1,000 individuals from 1987 to 2006 standardized to the age, race and sex distribution of the analytic cohort. [c]Models adjusted for study

Table 2.3 (continued) Multivariable-adjusted hazard ratios for cancer incidence by tertile of neutrophil count, stratified by race, sex and cigarette smoking status, in the ARIC study, 1987-2006

site (Jackson, Forsyth County, Minneapolis), race (white, black), sex, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (≤1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [d]Additionally adjusted for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user).

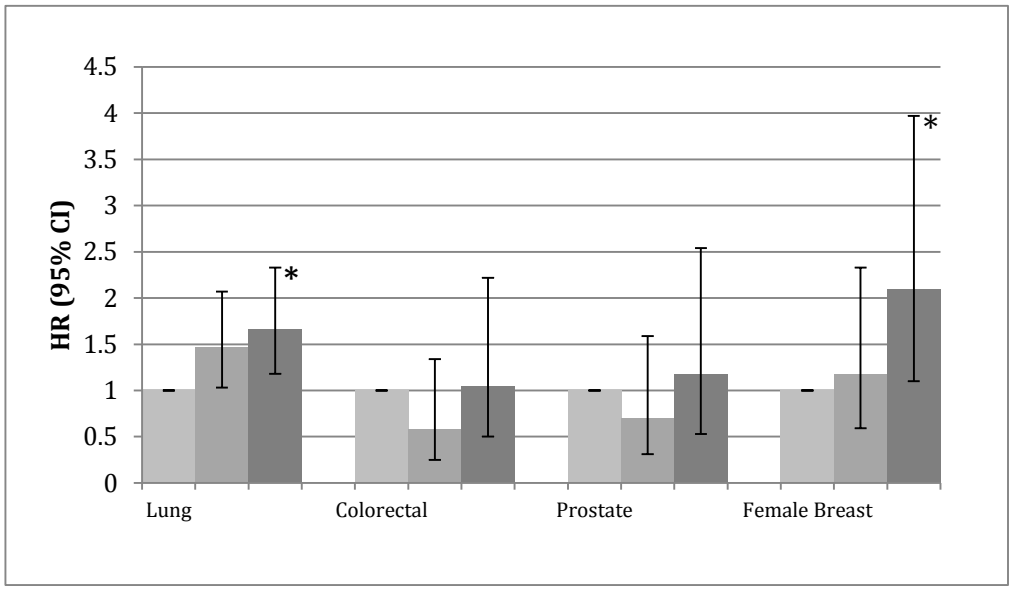

Figure 2.2 Multivariable-adjusted hazard ratios for site-specific cancer mortality by tertile of neutrophil count in the ARIC study, 1987-2008. Light gray=tertile 1; Medium gray=tertile 2; Dark gray=tertile 3. Neutrophil count tertiles defined based on race-specific cut-offs and combined across the analytic cohort. Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), sex, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke ($\leq$1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. Models of female breast cancer were additionally adjusted for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user). Asterisk indicates p-value<0.05.

Table 2.4 Multivariable-adjusted hazard ratios for cancer incidence and mortality by tertile of neutrophil count accounting for levels of lymphocytes, monocytes, eosinophils and basophils in the ARIC study, 1987-2008

Neutrophil Count by Tertile[a]	N	Person-Years	HR (95% CI) [b]
Cancer Incidence			
1	649	56010.3	1.00 (Reference)
2	702	53735.9	1.09 (0.96, 1.23)
3	759	51483.0	1.16 (1.02, 1.33)
P-Trend			0.02
Cancer Mortality			
1	234	69263.8	1.00 (Reference)
2	292	66405.1	1.17 (0.96, 1.42)
3	412	63086.7	1.38 (1.14, 1.69)
P-Trend			0.001

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Neutrophil count tertiles defined based on race-specific cut-offs and combined across the analytic cohort. [b]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), sex, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke ($\leq$1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, diabetes, lymphocyte count tertile ($\leq$1.65, 1.66-2.14, $\geq$2.15 x10^9 cells/L), monocyte count tertile ($\leq$0.272, 0.273-0.399, $\geq$0.400 x10^9 cells/L), eosinophil count tertile ($\leq$0.07, 0.07-0.174, $\geq$0.175 x10^9 cells/L) and basophil count category (<0.03, $\geq$0.03 x10^9 cells/L).

Appendix A. Chapter 2 supplemental tables

Table A.1 Leading types of cancer incidence and cancer mortality in the ARIC study, 1987-2008

	Incidence[a] (N)	**Mortality[b] (N)**
Lung	279	294
Colorectal	213	41
Female Breast	375	61
Prostate	482	41

[a]1987-2006. [b]1987-2008.

Table A.2 Baseline characteristics by tertile of neutrophil count in white and black ARIC participants, 1987-1989

	Neutrophil Count (10^9 cells/L)					
	Whites (N=6,620)			Blacks (N=3,717)		
	Tertile 1 (1.100-3.135)	**Tertile 2 (3.136-4.189)**	**Tertile 3 (4.200-9.180)**	**Tertile 1 (0.333-2.035)**	**Tertile 2 (2.040-3.024)**	**Tertile 3 (3.024-7.600)**
Total, N	2,221	2,196	2,203	1,242	1,236	1,239
Study Site[a], %						
Jackson, MS	0	0	0	93.9	85.8	83.5
Forsyth County, NC	45.3	46.7	46.6	5.6	14	15.7
Minneapolis, MN	54.7	53.3	53.4	0.6	0.2	0.8
Male, %	42.2	51.7	52.4	39.1	39.0	37.9
Age (years), Mean (SD)	54.0 (5.6)	54.3 (5.8)	54.1 (5.9)	53.4 (5.8)	53.4 (5.8)	53.7 (6.0)
Education, %						
<High school	8.4	11.0	13.3	42.3	42.2	40.6
High school or college graduate	43.0	45.3	46.5	27.8	28.4	29.1
Graduate school	48.7	43.7	40.2	29.8	29.5	30.3
Missing, N	2	3	3	2	5	2
BMI (kg/m^2), %						
<18.5	0.5	0.6	1.2	0.6	0.6	1.2
18.5-24.99	44.6	36.3	33.5	20.9	20.1	20.6
25.0 29.99	38.9	42.2	41.8	40.6	36.4	35.6
30.0-34.99	12.8	15.7	16.3	24.9	25.8	24.4
≥35.00	3.2	5.2	7.2	13.0	17.1	18.2
Missing, N	2	1	2	0	1	3
Waist Circumference (cm), Mean (SD)	92.8 (12.1)	95.7 (12.8)	96.9 (13.6)	97.6 (14.4)	99.8 (14.9)	100.4 (15.7)
Missing, N	0	0	1	0	0	2
Cigarette Use, %						
Never	50.6	39.6	23.6	53.2	44.5	41.0
Former	39.7	39.8	32.2	26.3	25.6	20.3

Table A.2 (continued) Baseline characteristics by tertile of neutrophil count in white and black ARIC participants, 1987-1989

	Neutrophil Count (10^9 cells/L)					
	Whites (N=6,620)			Blacks (N=3,717)		
	Tertile 1 (1.100-3.135)	**Tertile 2 (3.136-4.189)**	**Tertile 3 (4.200-9.180)**	**Tertile 1 (0.333-2.035)**	**Tertile 2 (2.040-3.024)**	**Tertile 3 (3.024-7.600)**
Current	9.8	20.6	44.3	20.6	26.9	38.8
Missing, N	2	1	1	1	2	1
Pack-Years[b], Mean (SD)	23.3 (21.7)	24.2 (21.9)	25.5 (22.0)	23.3 (21.9)	23.4 (21.6)	23.5 (21.7)
>1	58.5	66.3	75.8	75.3	75.9	77.9
Missing, N	18	9	10	7	10	8
Alcohol Intake[d], Mean (SD) (g/wk)	92.2 (94.8)	104.9 (119.0)	120.5 (136.7)	111.8 (119.5)	124.0 (165.1)	137.8 (189.4)
Missing, N	3	6	3	14	28	14
CVD[e], %	7.6	9.7	12.5	7.3	9.5	11.8
Hypertension[f], %	20.6	25.9	28.3	52.9	55.2	58.1
Missing, N	0	2	0	0	0	1
Diabetes[g], %	4.7	7.7	10.8	13.5	18.4	25.7
Missing, N	0	2	1	3	2	6
Aspirin used[h], %	53.7	52.5	54.7	26.6	30.9	31.4
Missing, N	7	7	10	22	25	11

Abbreviations: BMI, body mass index; SD, standard deviation; ETS, environmental tobacco smoke; CVD, cardiovascular disease. [a] Neutrophil count tertiles defined based on race-specific cut-offs and combined across the analytic cohort. [b]Participants from Washington County, MD were excluded from analyses due to substantial missing WBC subtype count data at this site. [c]Pack-years among ever smokers only. Pack-years was calculated as the average number of cigarettes smoked per day multiplied by the number of years of smoking and divided by 20. [d]ETS defined as the average number of hours per week of close contact with people when they are smoking. [e]Alcohol intake among participants who reported usually having at least one drink per week. [f]CVD defined as having a history of angina pectoris, coronary heart disease, intermittent claudication, or stroke. [g]Hypertension defined as use of any hypertensive medications, systolic blood pressure $\geq$140 mmHg, or diastolic blood pressure $\geq$90 mmHg. [h]Diabetes defined as having a fasting glucose level $\geq$126 mg/dl, non-fasting glucose level $\geq$200 mg/dl, a physician diagnosis of diabetes, or using sugar-lowering medications in two weeks prior to enrollment. [i]Aspirin use in two weeks prior to study enrollment.

Table A.3 Multivariable-adjusted hazard ratios for non-lung cancer incidence and mortality by tertile of neutrophil count and stratified by cigarette smoking status in the ARIC study, 1987-2008

	Current Smokers		Former Smokers		Never Smokers	
Neutrophil Count by Tertiles[a]	**Age-Standardized Rate[b]**	**HR (95% CI)[c]**	**Age-Standardized Rate[b]**	**HR[c] (95% CI)**	**Age-Standardized Rate[b]**	**HR (95% CI)[c]**
Cancer Incidence						
1	159.2	1.00 (Reference)	200.3	1.00 (Reference)	167.9	1.00 (Reference)
2	180.2	1.10 (0.83, 1.45)	182.7	0.91 (0.76, 1.10)	180.4	1.08 (0.91, 1.28)
3	181.8	1.21 (0.93, 1.57)	187.5	0.96 (0.79, 1.17)	169.7	1.07 (0.88, 1.30)
P-Trend		0.13		0.66		0.43
Cancer Mortality						
1	79.1	1.00 (Reference)	69.4	1.00 (Reference)	40.4	1.00 (Reference)
2	71.8	0.81 (0.53, 1.22)	57.1	0.90 (0.64, 1.25)	54.6	1.33 (0.96, 1.85)
3	85.8	1.16 (0.80, 1.68)	81.7	1.36 (0.98, 1.88)	59.6	1.52 (1.07, 2.16)

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Neutrophil count tertiles defined based on race-specific cut-offs and combined across the analytic cohort. [b]Incidence rate per 1,000 individuals from 1987 to 2006 standardized to the age, race and sex distribution of the analytic cohort. Mortality rate per 1,000 individuals from 1987 to 2008 standardized to the age, race and sex distribution of the analytic cohort. [c]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), sex, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (≤1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes.

Table A.4 Correlations of baseline counts of total WBC and subtype counts in ARIC, 1987-1989

	Total WBC	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Total WBC	1.0000					
Neutrophil	0.8649	1.0000				
Lymphocyte	0.4764	0.0609	1.0000			
Monocyte	0.4562	0.3299	0.1764	1.0000		
Eosinophil	0.1839	0.474	0.1268	0.0091	1.0000	
Basophil	0.1473	0.0808	0.0837	-0.0408	0.3446	1.0000

Table A.5 Multivariable-adjusted hazard ratios for cancer incidence and mortality by quartile of total WBC count in the ARIC study, 1987-2008

WBC Count ($x10^9$ cells/L)	HR[a] (95% CI)
Cancer Incidence	
Quartile 1 (3.3 – 4.8)	1.00 (Reference)
Quartile 2 (4.9 – 5.8)	0.95 (0.84, 1.07)
Quartile 3 (5.9 – 7.0)	1.02 (0.90, 1.16)
Quartile 4 (7.1 – 10.7)	1.07 (0.94, 1.22)
P-Trend	0.20
Cancer Mortality	
Quartile 1 (3.3 – 4.8)	1.00 (Reference)
Quartile 2 (4.9 – 5.8)	1.14 (0.93, 1.40)
Quartile 3 (5.9 – 7.0)	1.40 (1.14, 1.71)
Quartile 4 (7.1 – 10.7)	1.47 (1.20, 1.81)
P-Trend	<0.001

Abbreviations: WBC, white blood cell; HR, hazard ratio; CI, confidence interval. [a]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), sex, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (≤1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes.

Chapter 3. Lymphocyte count, cancer incidence and mortality in the Atherosclerosis Risk in Communities (ARIC) study: Differential associations in men and women

Abstract

The tumor immunosurveillance hypothesis posits a role for host immune factors in the identification and clearance of premalignant and malignant cells. Lymphocytes, which constitute the adaptive immune response, may have an important role in this anti-tumoral mechanism. We hypothesized that circulating levels of pre-diagnostic lymphocyte count would be inversely associated with subsequent cancer incidence and mortality. To evaluate this, we used data from the Atherosclerosis Risk in Communities (ARIC) study. Participants included men and women with total white blood cell (WBC) counts within the normal reference range and no history of cancer at baseline (N=10,316). Total WBC and subtype counts were measured at baseline (1987-1989) and cancer incidence and mortality were available through 2006 and 2008, respectively. Cox proportional hazards models were used to estimate the multivariable-adjusted hazard ratios (HR) and 95% confidence intervals (CI) by tertile of lymphocyte count in men and women, separately. During follow-up, a total of 2,153 incident primary cancers and 956 cancer deaths occurred. In men, lymphocyte count was not associated with total cancer incidence. However, in cancer incidence site-specific analyses, high lymphocyte count was associated with an increased risk of prostate cancer (HR: 1.31, 95% CI: 1.03, 1.66) and there was a non-linear reduced risk of colorectal cancer. After excluding cases of prostate cancer, the highest tertile of lymphocyte count was associated with 25% reduced risk of cancer incidence (HR: 0.75, 95% CI: 0.62, 0.91). An inverse association with non-prostate cancer incidence persisted after mutual adjustment for other WBC subtype counts, among men with lymphocyte counts within the normal reference range, and after excluding cancer cases within the first five years of follow-up. In women, the highest

tertile of lymphocyte count was associated with an increased risk of cancer mortality, overall (HR: 1.40, 95% CI: 1.07, 1.82), and among current smokers. In men, high lymphocyte count was associated with a reduced risk of cancer incidence, excluding cases of prostate cancer, while in women, high lymphocyte count was associated with an increased risk of cancer mortality. Future studies are necessary to explore the differential effects of lymphocytes on carcinogenesis by sex.

Introduction

The tumor immunosurveillance concept, proposed in the late 1950's, posits a role for the host immune system in identifying and clearing premalignant and malignant cells (1). Recently, this hypothesis has received a resurgence of interest in the literature (2-4), in part, due to the accumulating evidence from in vivo studies linking impairment of lymphocyte function with the increased development of cancer (2, 3, 5-9). These findings lend more definitive support for the tumor immunosurveillance process and suggest a possible role for lymphocytes in this anti-tumoral mechanism.

Lymphocytes, a subtype of total white blood cells (WBC), include B and T cells, which constitute the cellular basis of the adaptive immune response. Additionally, the third broad type of lymphocytes, natural killer (NK) cells, are a component of the innate immune system (10). In in vitro studies, T cells and NK cells have been shown to exert anti-tumoral effects through the recognition of tumor antigens, direct cytotoxic effects, and the production of certain cytokines (2, 9, 11, 12). However, certain subsets of T cells may also have the capacity to exert pro-tumoral effects (13).

Few observational studies have examined the relationship between lymphocytes and tumor development and progression. In the clinical setting, higher concentrations of NK cells and T lymphocytes in the tumor infiltrate have been associated with prolonged survival of colorectal, ovarian and gastric cancers (14-18), while other lymphocyte subsets have been associated with poorer prognosis (13, 19-21). Additionally, circulating

neutrophil to lymphocyte ratio (NLR) at diagnosis has been inversely associated with cancer survival in many studies (22-24).

In a prospective epidemiological study of healthy Japanese adults, moderate and high NK cell cytotoxic activity, a measure of lymphocyte function, were associated with reduced risks of subsequent total cancer incidence over an 11 year period (25). To date, only one previous study has evaluated the association between circulating pre-diagnostic lymphocyte count and subsequent cancer incidence. In this study, no association was found based on age- and sex-adjusted models only (26). To expand upon these findings, we prospectively evaluated the relationship between circulating lymphocyte count and cancer incidence and mortality in the Atherosclerosis Risk in Communities (ARIC) study, a large, prospective, community-based cohort. We hypothesized that higher levels of pre-diagnostic lymphocyte count would be associated with a subsequent reduced risk of cancer incidence and mortality, providing further support for the tumor immunosurveillance concept.

Materials and Methods

Study population

This study was conducted in the ARIC study, an ongoing, prospective cohort initiated between 1987 and 1989 to investigate the etiology of atherosclerosis and its sequelae. Men and women (N=15,792), ages 45 to 64 years, were enrolled from four U.S. communities, Forsyth County, NC; Jackson, MS; Washington County, MD; and suburban Minneapolis, MN. Participants were identified using probability sampling in Minneapolis

and Washington County, while in Jackson, blacks were exclusively recruited and in Forsyth County blacks were oversampled (27). Participants from Washington County were not included in these analyses as baseline WBC subtype counts were missing for more than 85% of participants (28). At enrollment, participants provided a blood sample and reported information on sociodemographic factors, medical history, reproductive history, physical activity, alcohol and tobacco use and other lifestyle behaviors via standardized questionnaires.

The analytic cohort included both men and women that met the following eligibility criteria: (1) not residing in Washington County, MD (N=11,792); (2) no personal history of cancer, excluding cases of non-melanoma skin cancer (N=11,175); (3) white or black race (N=11,140); (4) total WBC count within two standard deviations of the mean in whites and blacks, separately (N=10,351); (5) not missing baseline lymphocyte count (N=10,339); and (6) not missing information on cancer incidence (N=10,177) or mortality (N=10,316).

Exposure ascertainment

Total and differential WBC counts were measured at baseline and three years later at Visit 2 (1990-1992). In the main analyses, only baseline WBC subtype counts were used. Following venipuncture, samples were stored at 4°C and within 24 hours total WBC count was measured using automated particle counters in local, independent clinical laboratories. Subtypes were measured as a proportion of total WBCs and counts were then calculated by multiplying the subtype proportion by the total WBC count. Based on

repeat testing of individuals conducted one to two weeks apart, reliability coefficients for total WBC count were estimated to be greater than 0.96 for each laboratory (29, 30).

Outcome ascertainment

The incidence of a first primary cancer, including date of diagnosis and site of cancer, was ascertained from study initiation through December 31, 2006 (30, 31). Cancer incidence was primarily identified by linkage to well-established state and/or county cancer registries that have a high completeness (≥90%) of cancer data (30). Hospital surveillance was used to identify cancer cases in Jackson prior to establishment of the Mississippi state cancer registry in 1996 (32), and all additional cancer cases for the other study sites (30, 33). At present, data on stage at diagnosis, cancer subtype and treatment are not consistently available for all cancers.

Vital status was available through December 31, 2008. Deaths were identified through contact with relatives, physician or designated contact, or through a search of obituaries, funeral and hospital records, death certificates and the National Death Index (NDI). The date and cause of death were confirmed by death certificate for all reported deaths. Cause of death was coded using the Ninth International Statistical Classification of Diseases and Related Health Problems (ICD-9) for deaths through 1998 and ICD-10 for all subsequent deaths. Cause-specific mortality from death certificate was available for 98% of decedents (34).

Assessment of covariates

Participants reported their highest education attainment, regular alcohol use, and intake of aspirin in the two weeks prior to baseline study visit. They also provided information on current cigarette smoking status and, if applicable, the average number of years of smoking and cigarettes smoked per day. These values were used to calculate pack-years ([cigarettes per day*years smoking]/20 cigarettes per pack) among ever smokers. Exposure to environmental tobacco smoke (ETS) was defined as being in close proximity to smokers for more than 1 hour per week (35, 36). Body mass index (BMI), calculated as weight (kg)/[height(m)]^2, and waist circumference were collected by trained technicians at baseline study visit. A history of cardiovascular disease (CVD) was defined as having a prior diagnosis of angina pectoris, coronary heart disease, intermittent claudication or stroke. Participants were categorized as having hypertension if they reported use of any hypertensive medications or if they had systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Diabetes was defined as having a fasting glucose level ≥126 mg/dl, a non-fasting glucose level ≥200 mg/dl, a self-reported physician diagnosis of diabetes, or use of blood sugar lowering medications in the two weeks prior to enrollment. Women were categorized as being premenopausal if they had a menstrual cycle within two years of baseline or postmenopausal (37). Women missing information on menopausal status were categorized as being postmenopausal if they were 55 years of age or older. Postmenopausal hormone use was categorized as current, former or never (37).

Statistical analyses

Baseline descriptive characteristics were compared by tertile of lymphocyte count using the chi-square test for categorical variables or ANOVA for continuous variables. Risk analyses are presented separately for men and women as we observed significant differences in the associations between lymphocyte count and cancer incidence and mortality by sex. Cox proportional hazards models were used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for cancer incidence and mortality by tertile of lymphocyte count. Tests for linear trend across categories of lymphocyte count were calculated by introducing the median value of each tertile as a continuous variable into models. To explore the potential of non-linearity, we also modeled lymphocyte count continuously using restricted cubic splines with knots at the 10^{th}, 50^{th} and 90^{th} percentiles. In spline models, excluding values in the top and bottom 1% to account for the effects of outliers did not appreciably alter risk estimates. Additional analyses were performed examining the association between tertile of NLR and cancer incidence and mortality. Tertiles of NLR were defined separately for whites and blacks, given that blacks have significantly lower levels of circulating neutrophils (38, 39), and combined across the total analytic cohort. In analyses of cancer incidence, follow-up time was accrued from age at baseline blood draw, with the origin defined as age 40 years and staggered late entries for persons over age 40 at baseline, to the first of the following events: (1) age at first primary cancer diagnosis, (2) age at death, or (3) age at end of follow-up (December 31, 2006). In analyses of cancer mortality, individuals were followed from age at baseline blood draw to (1) age at death or (2) age at end of follow-up (December 31, 2008). In models in which time accrued from year at baseline blood draw to (1) year at first

primary cancer diagnosis or cancer death, (2) year at other death, or (3) year at end of follow-up, similar HRs were estimated and are not presented here.

Cox multivariable models included the baseline covariates, race (white, black), study site (Jackson, MS, Forsyth County, Minneapolis, MN), BMI (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m^2), waist circumference (continuous), education attainment (<high school diploma, high school diploma, >high school or college graduate, graduate school), cigarette smoking status (never, former, current), pack-years (continuous), ETS (≤1 hour/week, >1 hour/week), alcohol intake (g/wk), aspirin use in two weeks prior to blood draw (yes/no), and medical history of CVD (yes/no), hypertension (yes/no), and diabetes (yes/no). In women, the additional covariates, menopausal status and HRT use (premenopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user) were included in models. Additionally, mutual adjustment by counts of the other WBC subtypes (neutrophils, monocytes, basophils and eosinopohils) was performed in both men and women. Missing pack-year and waist circumference information was replaced with the median value. Effect modification between lymphocyte count (and NLR) and select covariates known to modulate the host immune response, including sex, race, cigarette smoking status, BMI, menopausal status and aspirin use, was assessed by introducing a cross-product term in models and using the Wald test to test for statistical significance. The proportional hazards assumption was assessed by introducing an interaction term between lymphocyte count tertile (and NLR tertile) and follow-up time into models. In all cases, the interaction term was not statistically significant, confirming this assumption. Rates of

cancer incidence and cancer mortality were standardized to the age and race distribution of the analytic cohort.

Cancer site-specific analyses were conducted in models of cancer incidence and cancer mortality for the four most common cancers (i.e., female breast cancer, colorectal cancer, lung cancer, and prostate cancer). Additionally, the following sensitivity analyses were performed: (1) excluding incident cancer cases and deaths within one and five years from baseline blood draw in order to address any bias due to reverse causation; (2) excluding individuals with lymphocyte counts outside the normal reference range, 1.5 to 3.0 x 10^9 cells/L, to more rigorously restrict analyses to individuals with no underlying acute or chronic immune conditions; (3) removing ever smokers and users of aspirin in the two weeks prior to baseline; and (4) using lymphocyte count measured at Visit 2 as the exposure of interest in a subset of participants with available data (N=6,582); In these analyses time at risk was initiated at age at Visit 2. Finally, in cancer mortality analyses, sub-distribution hazard ratios were estimated using the Fine and Gray approach to account for the issue of competing risks (40). All analyses were conducted using STATA version 11.2 (Stata Corporation, College Station, TX, 2012).

Results

Table 3.1 presents the distribution of baseline characteristics by tertile of lymphocyte count. The median lymphocyte count in the analytic cohort was 1.89 x 10^9 cells/L (interquartile range: 1.54, 2.30 x 10^9 cells/L). Individuals with higher circulating lymphocyte count were more likely to be female, black, less educated and have a higher

BMI and mean waist circumference. They were also more likely to have a medical history of CVD, hypertension and diabetes and reported more frequent use of tobacco and alcohol and greater exposure to ETS. We observed a similar distribution of the baseline variables by lymphocyte tertile in males and females, separately (Appendix B, Table B.1).

Cancer incidence

Between 1987 and 2006, over a median follow-up time of 18.1 years, 1,163 and 990 incident primary cancer cases were ascertained in men and women, respectively. There was a significant interaction between lymphocyte count tertile and sex in models of cancer incidence (all p-interaction terms$\leq$0.009). Figure 3.1 presents the multivariable-adjusted HR for total and site-specific cancer incidence by tertile of lymphocyte count in men. Overall, there was no significant association between lymphocyte count tertile and cancer incidence. However, in cancer incidence site-specific analyses, men in the middle tertile of lymphocyte count had a 48% (HR: 0.52, 95% CI: 0.32, 0.86) reduced risk of colorectal cancer although there was no association for the highest tertile of lymphocyte count compared to the lowest tertile (HR: 0.77, 95% CI: 0.48, 1.23) (Figure 3.1). In models of prostate cancer incidence, there was a positive, linear association with lymphocyte count tertile ($HR_{\text{tertile 3 v tertile 1}}$: 1.31, 95% CI: 1.03, 1.66, p-trend=0.02). There was no significant association between lymphocyte count tertile and lung cancer incidence.

Given the differential association between lymphocyte count tertile and cancer incidence by organ site, we evaluated the risk of cancer incidence in men excluding cases of prostate cancer (Table 3.2). There was a significant reduced risk of non-prostate cancer incidence among men in both the middle (HR: 0.68, 95% CI: 0.56, 0.82) and highest tertile (HR: 0.75, 95% CI: 0.62, 0.91) of lymphocyte count, relative to the lowest tertile. This association held across strata of race and current cigarette use (Table 3.2, all p-interaction terms $\geq$0.2), and after excluding incident cancers diagnosed within one and five years of follow-up. Additionally, there was no effect modification by age, BMI, or recent aspirin use. Mutual adjustment with the other WBC subtypes did not appreciably alter risk estimates (Appendix B, Table B.2). In analyses restricted to the normal reference range of lymphocyte count, 1.5 to 3.0 x 10^9 cells/L, a significantly reduced risk of non-prostate cancer incidence persisted in men in the middle tertile of lymphocyte count (HR: 0.75, 95% CI: 0.59, 0.94) and there was a non-significant reduced risk in men in the highest tertile (HR: 0.83, 95% CI: 0.66, 1.05). Similarly, after excluding aspirin users, a significant reduced risk of non-prostate cancer incidence persisted in the middle tertile of lymphocyte count only. When using lymphocyte count measured at Visit 2, a non-significant reduced risk of non-prostate cancer incidence was found for the middle (HR: 0.84, 95% CI: 0.67, 1.06) and highest (HR: 0.86, 95% CI: 0.68, 1.09) tertiles of lymphocyte count, compared to the lowest. In spline regression models, there was a reduced risk of non-prostate cancer incidence with increasing values of lymphocyte count up to 2.3 x10^9 cells/L, at which point the risk plateaus (Figure 3.2, p-linearity=0.004).

Among women, there was no association between lymphocyte count tertile and cancer incidence, overall, or in analyses stratified by race, menopausal status and current cigarette use (all p-interaction terms ≥0.09) (Table 3.3). Additionally, no significant associations were found for the incidence of major female cancer types, including breast, lung and colorectal cancer (Figure 3.3).

Cancer mortality

During a median follow-up time of 21.4 years, between 1987 and 2008, 539 and 400 cancer deaths occurred in men and women, respectively. We observed a significant interaction between lymphocyte count tertile and sex in models of cancer mortality (all p-interaction terms<0.05). Figure 3.4 presents multivariable-adjusted risk estimates of total and site-specific cancer mortality in men. There was no significant association between lymphocyte count and lung, colorectal or prostate cancer mortality. However, in models of total cancer mortality, there was a slight inverse association between the middle tertile of lymphocyte count and cancer mortality, although this estimate did not achieve statistical significance. Excluding cases of prostate cancer mortality did not alter risk estimates (Table 3.4). Additionally, there was no effect modification by race, cigarette smoking status, BMI or asprin use (all p-interaction terms ≥0.4).

In women, the highest tertile of lymphocyte count was associated with a 40% (HR: 1.40, 95% CI: 1.07, 1.82, p-trend=0.02) increased risk of cancer mortality (Table 3.5). A significant association persisted after excluding cancer deaths within one and five years of baseline and after excluding participants with lymphocyte counts outside the normal

reference range (HR: 1.35, 95% CI: 0.99, 1.83). After further adjustment for the other WBC subtypes, a positive association between lymphocyte count and cancer mortality persisted, however this association was no longer linear (Appendix B, Table B.2). Similar findings were estimated in pre/peri-menopausal and postmenopausal women (Table 3.5). In analyses stratified by cigarette use, a positive association between the highest tertile of lymphocyte count and cancer mortality was observed in current smokers, but not in former and never smokers, although there was no significant interaction term between lymphocyte count tertile and smoking status (Table 3.5; all p-interaction terms $\geq$0.3). Additionally, there was no association between lymphocyte count and cancer mortality in women who did not use aspirin in the two weeks prior to blood draw. There was no evidence of a statistical interaction between the highest tertile of lymphocyte count and race, menopausal status, age, BMI or recent aspirin intake (all p-interaction terms $\geq$0.2). In the analysis using lymphocyte count measured at Visit 2, lymphocyte count was not significantly associated with cancer mortality (HR: 1.24, 95% CI: 0.88, 1.76).

In women, lymphocyte count tertile was not associated with colorectal or breast cancer mortality but there was a suggestive increased risk of lung cancer mortality in women with high lymphocyte count compared to low (HR: 1.62, 95% CI: 0.93, 2.84, p-trend=0.09) (Figure 3.5). A suggestive positive association was also found between the highest tertile of lymphocyte count and non-lung cancer mortality (HR: 1.32, 95% CI: 0.97, 1.80, p-trend=0.08).

Finally, lymphocyte count tertile was not associated with CVD or non-cancer mortality in men or women. In competing risks analyses, a significant association persisted between the highest tertile of lymphocyte count and cancer mortality among women (sub-distribution HR: 1.40, 95% CI: 1.08, 1.83).

NLR

There was no evidence of effect modification between NLR and sex in models of cancer incidence or mortality (all p-interactions $\geq$0.6). Furthermore, there were no associations between tertile of NLR and cancer incidence or mortality, overall, or in strata of sex, race, or cigarette smoking status (Appendix B, Table B.3).

Discussion

To our knowledge, this is the first study to report a differential association between lymphocyte count and cancer incidence and mortality by sex. In this large prospective study, men with high lymphocyte counts had a reduced risk of developing cancer, with the exception of prostate cancer. The significant inverse association with non-prostate cancer incidence remained in analyses limited to incident cancers diagnosed at least five years post baseline blood draw to account for the presence of subclinical disease, among never smokers and non-users of aspirin, and after mutual adjustment for the other WBC subtypes. In cancer incidence site-specific analyses, the reduced risk of cancer incidence was strongest for colorectal cancer, while high lymphocyte count was significantly associated with an increased risk of prostate cancer incidence. In contrast, among women, high lymphocyte count was associated with an increased risk of total cancer mortality,

but not cancer incidence. This positive association with cancer mortality, however, did not persist among never smokers or non-users of aspirin.

Our finding of an inverse association between circulating lymphocyte count and non-prostate cancer incidence in men lend credence to the tumor immunosurveillance concept. Moreover, our results support the hypothesis that lymphocytes are involved in this anti-tumoral mechanism. To date, the majority of the evidence for the tumor immunosurveillance concept comes from studies conducted in mice models (2, 41). Congruent with our findings, these studies report an increased risk of spontaneous and chemically induced tumors in mice with impaired lymphocyte function (5-8). Additionally, based on in vitro and in vivo studies, specific lymphocyte subsets, including NK cells and cytotoxic T cells, have the capacity to both recognize tumor antigens and kills tumor cells, directly or indirectly, through the production of cytokines (9).

The present study expands upon these laboratory findings by demonstrating that inter-individual variations of circulating, pre-diagnostic lymphocyte count within the normal reference range have implications for cancer risk. Notably, although circulating lymphocyte count is not a direct measure of functional immunity, our findings are consistent with prior studies, which have measured lymphocyte function in healthy adult populations. Specifically, in several cross-sectional studies, NK cell-mediated cytotoxicity and lymphocyte proliferation were lower in cancer-free individuals with a familial history of cancer compared to controls with no family history of cancer (42-46),

and in a longitudinal study of 3,625 Japanese men and women, high NK cytotoxic activity was associated with a 41% reduction in total cancer risk over an 11 year period of follow-up (25).

To our knowledge, only one previous study has evaluated the association between circulating lymphocyte count and subsequent cancer incidence. In contrast to our findings, in this study of 669 Danish men and women, no association was found in sex- and age-adjusted models (26). However, effect modification by sex was not evaluated in this study, and other factors such as race, BMI and cigarette smoking status were not accounted for in models.

In men, the effect of circulating lymphocyte count on cancer risk differed by cancer site. While elevated lymphocyte count was associated with a reduced risk of colorectal cancer incidence, we observed a positive association between lymphocyte count and prostate cancer risk. Interestingly, epidemiological studies evaluating the association between allergies, a Th2 mediated condition, and cancer risk, have also noted differences in the risk of prostate cancer compared to other cancer types. Having a history of allergies has been associated with a reduced risk of certain cancers, particularly pancreatic cancer and gliomas (47, 48), and an *increased* risk of prostate cancer in many (49-55) but not all prospective studies (56, 57). However, the mechanism underlying this differential effect on the prostate remains unknown.

It is also possible that the positive association between lymphocyte count and prostate cancer incidence reflect a screening bias, such that men with higher lymphocyte counts are more likely to receive PSA screening. In the present study, we were not able to evaluate this, as information on PSA screening was not collected. However, this explanation is not entirely convincing given that our analyses were restricted to lymphocyte counts within the normal reference range and differential screening patterns within this range would not be expected. Additionally, the differential association with prostate cancer incidence seems to be specific to lymphocytes; Similar findings have not been observed in individuals with elevated levels of other circulating immune markers (58-60), further suggesting that the observed increased risk of prostate cancer is not an artifact due to differential PSA screening.

Unlike in men, in women, high levels of pre-diagnostic lymphocyte count were associated with a 40% *increased* risk of cancer mortality. However, in stratified analyses, this significant association did not persist among never smokers or in women who did not take aspirin in the two weeks prior to baseline. Thus, we cannot exclude the possibility of residual confounding by cigarette use, despite having adjusted for pack-years, and aspirin use. In particular, cigarette use is a strong positive confounder in our analyses, associated with both increased lymphocyte counts and increased cancer incidence and mortality. Additionally, in this study, detailed information on aspirin use, including duration of use, was not collected. Alternatively, the lack of an association in these strata may be due the limited sample size in these subgroups. Thus, larger studies are necessary to clarify the association between lymphocytes and cancer mortality in women.

The observed sex-related differences in lymphocyte activity may reflect differences in the absolute and relative counts of lymphocyte subsets in men and women. It is hypothesized that the effect of androgens on thymocyte cells alters the make-up of peripheral T cells (61). Consistent with this hypothesis, many studies have reported higher levels of CD3+ and CD4+ cells, higher ratios of CD3+/CD4+ and CD4+/CD8+, and lower levels of NK cells in healthy adult women, compared to men (62-66). Given that CD8+ cytotoxic T lymphocytes and NK cells are critical components in the tumor immunosurveillance response (9), this shift in the distribution of T lymphocyte subsets may explain the potential anti-tumoral effect of lymphocytes in men and pro-tumoral effect in women. In the present study, while the majority of circulating lymphocytes are T cells, information on T cell subsets was not available. Future studies are warranted to evaluate this hypothesis.

Our study findings must be interpreted in light of certain additional limitations. By utilizing a one-time measurement of lymphocyte count, we did not account for time-varying changes in the level of this marker or intra-individual variation. However, in ARIC, peripheral lymphocyte count was measured at two time points, baseline and three years later, at Visit 2. The correlation between continuous values of lymphocyte count over time was 0.70 and 0.67 in men and women, respectively, and the agreement across tertiles was 61.3% and 58.4% in men and women. These comparisons indicate that lymphocyte count is fairly stable in this population of healthy adults. Indeed, in sensitivity analyses using lymphocyte counts measured at Visit 2, a reduced risk of non-

prostate cancer incidence persisted in men with high lymphocyte count, although the positive association between lymphocyte count and cancer mortality in women was no longer present. In the present study, we were also not able to explore the effect of lymphocytes on tumor stage, grade or treatment, as this information was not available. This study also has several strengths. The ARIC study is a prospective, population-based cohort with over 20 years of follow-up for cancer incidence and mortality. Additionally, detailed information was available on a wide-array of socio-demographic characteristics, lifestyle risk factors and medical history.

In summary, among men with total WBC counts within the normal reference range, high lymphocyte count was associated with a reduced risk of cancer incidence, excluding prostate cancer, and an increased risk of prostate cancer incidence. In contrast, in women, high lymphocyte count was associated with an increased risk of total cancer mortality, however this association may reflect residual confounding due to factors including cigarette smoking and aspirin use. Based on these novel findings, circulating lymphocyte count may be a novel marker of the tumor immunosurveillance response in men. Furthermore, we hypothesize that the differences in lymphocyte-mediated carcinogenesis by sex and organ site may reflect differences, due to hormonal effects, in the absolute and relative levels of circulating and localized lymphocyte subsets. Future studies are necessary to validate our study findings and to investigate this hypothesis.

Acknowledgements

The authors thank the staff and participants of the ARIC study for their important contributions and the ARIC Cancer Working Group for their feedback on this work.

References

1. Burnet M. Cancer: a biological approach. III. Viruses associated with neoplastic conditions. IV. Practical applications. Br Med J. 1957;1:841-7.
2. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Adv Immunol. 2006;90:1-50.
3. Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature. 2012;482:400-4.
4. Joseph CG, Darrah E, Shah AA, Skora AD, Casciola-Rosen LA, Wigley FM, et al. Association of the autoimmune disease scleroderma with an immunologic response to cancer. Science. 2014;343:152-7.
5. Girardi M, Oppenheim DE, Steele CR, Lewis JM, Glusac E, Filler R, et al. Regulation of cutaneous malignancy by gammadelta T cells. Science. 2001;294:605-9.
6. Gao Y, Yang W, Pan M, Scully E, Girardi M, Augenlicht LH, et al. Gamma delta T cells provide an early source of interferon gamma in tumor immunity. J Exp Med. 2003;198:433-42.
7. Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, et al. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature. 2001;410:1107-11.
8. Shinkai Y, Rathbun G, Lam KP, Oltz EM, Stewart V, Mendelsohn M, et al. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. Cell. 1992;68:855-67.
9. Nakachi K, Hayashi T, Imai K, Kusunoki Y. Perspectives on cancer immuno-epidemiology. Cancer Sci. 2004;95:921-9.
10. Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J. Harrison's Principles of Internal Medicine. 18 ed: McGraw-Hill; 2011.
11. Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Sato H, et al. Natural killer-like nonspecific tumor cell lysis mediated by specific ligand-activated Valpha14 NKT cells. Proc Natl Acad Sci U S A. 1998;95:5690-3.
12. Wu J, Lanier LL. Natural killer cells and cancer. Adv Cancer Res. 2003;90:127-56.
13. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140:883-99.
14. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313:1960-4.
15. Laghi L, Bianchi P, Miranda E, Balladore E, Pacetti V, Grizzi F, et al. CD3+ cells at the invasive margin of deeply invading (pT3-T4) colorectal cancer and risk of post-surgical metastasis: a longitudinal study. Lancet Oncol. 2009;10:877-84.
16. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A. 2005;102:18538-43.

17. Coca S, Perez-Piqueras J, Martinez D, Colmenarejo A, Saez MA, Vallejo C, et al. The prognostic significance of intratumoral natural killer cells in patients with colorectal carcinoma. Cancer. 1997;79:2320-8.
18. Ishigami S, Natsugoe S, Tokuda K, Nakajo A, Che X, Iwashige H, et al. Prognostic value of intratumoral natural killer cells in gastric carcinoma. Cancer. 2000;88:577-83.
19. Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest. 2007;117:1175-83.
20. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med. 2004;10:942-9.
21. McArdle PA, Canna K, McMillan DC, McNicol AM, Campbell R, Underwood MA. The relationship between T-lymphocyte subset infiltration and survival in patients with prostate cancer. Br J Cancer. 2004;91:541-3.
22. Cho H, Hur HW, Kim SW, Kim SH, Kim JH, Kim YT, et al. Pre-treatment neutrophil to lymphocyte ratio is elevated in epithelial ovarian cancer and predicts survival after treatment. Cancer Immunol Immunother. 2009;58:15-23.
23. Sharaiha RZ, Halazun KJ, Mirza F, Port JL, Lee PC, Neugut AI, et al. Elevated preoperative neutrophil:lymphocyte ratio as a predictor of postoperative disease recurrence in esophageal cancer. Ann Surg Oncol. 2011;18:3362-9.
24. Walsh SR, Cook EJ, Goulder F, Justin TA, Keeling NJ. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. J Surg Oncol. 2005;91:181-4.
25. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet. 2000;356:1795-9.
26. Sajadieh A, Mouridsen MR, Selmer C, Intzilakis T, Nielsen OW, Haugaard SB. Monocyte number associated with incident cancer and mortality in middle-aged and elderly community-dwelling Danes. Eur J Cancer. 2011;47:2015-22.
27. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. Am J Epidemiol. 1989;129:687-702.
28. Prizment AE, Anderson KE, Visvanathan K, Folsom AR. Inverse association of eosinophil count with colorectal cancer incidence: atherosclerosis risk in communities study. Cancer Epidemiol Biomarkers Prev. 2011;20:1861-4.
29. Nieto FJ, Szklo M, Folsom AR, Rock R, Mercuri M. Leukocyte count correlates in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol. 1992;136:525-37.
30. Ahmed RL, Schmitz KH, Anderson KE, Rosamond WD, Folsom AR. The metabolic syndrome and risk of incident colorectal cancer. Cancer. 2006;107:28-36.
31. Prizment AE, Anderson KE, Visvanathan K, Folsom AR. Association of inflammatory markers with colorectal cancer incidence in the atherosclerosis risk in communities study. Cancer Epidemiol Biomarkers Prev. 2011;20:297-307.
32. Mississippi Cancer Registry Reporting Manual Revised 2011. Manual2011.
33. Prizment AE, Folsom AR, Dreyfus J, Anderson KE, Visvanathan K, Joshu CE, et al. Plasma C-reactive protein, genetic risk score, and risk of common cancers in the Atherosclerosis Risk in Communities study. Cancer Causes Control. 2013;24:2077-87.

34. Rose KM, Eigenbrodt ML, Biga RL, Couper DJ, Light KC, Sharrett AR, et al. Orthostatic hypotension predicts mortality in middle-aged adults: the Atherosclerosis Risk In Communities (ARIC) Study. Circulation. 2006;114:630-6.
35. Howard G, Wagenknecht LE, Burke GL, Diez-Roux A, Evans GW, McGovern P, et al. Cigarette smoking and progression of atherosclerosis: The Atherosclerosis Risk in Communities (ARIC) Study. JAMA. 1998;279:119-24.
36. Kan H, Heiss G, Rose KM, Whitsel EA, Lurmann F, London SJ. Prospective analysis of traffic exposure as a risk factor for incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study. Environ Health Perspect. 2008;116:1463-8.
37. Nabulsi AA, Folsom AR, Szklo M, White A, Higgins M, Heiss G. No association of menopause and hormone replacement therapy with carotid artery intima-media thickness. Atherosclerosis Risk in Communities (ARIC) Study Investigators. Circulation. 1996;94:1857-63.
38. Haddy TB, Rana SR, Castro O. Benign ethnic neutropenia: what is a normal absolute neutrophil count? J Lab Clin Med. 1999;133:15-22.
39. Lim EM, Cembrowski G, Cembrowski M, Clarke G. Race-specific WBC and neutrophil count reference intervals. Int J Lab Hematol. 2010;32:590-7.
40. Fine J, Gray R. A proportional Hazards Model for the Subdistribution of Competing Risk. Journal of the American Statistical Association. 1999;94:496-509.
41. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity. 2004;21:137-48.
42. Hersey P, Edwards A, Honeyman M, McCarthy WH. Low natural-killer-cell activity in familial melanoma patients and their relatives. Br J Cancer. 1979;40:113-22.
43. Strayer DR, Carter WA, Mayberry SD, Pequignot E, Brodsky I. Low natural cytotoxicity of peripheral blood mononuclear cells in individuals with high familial incidences of cancer. Cancer Res. 1984;44:370-4.
44. Hershey P, Edwards A, Milton GW, McCarthy WH. Relationship of cell-mediated cytotoxicity against melanoma cells to prognosis in melanoma patients. Br J Cancer. 1978;37:505-13.
45. Shevde LA, Joshi NN, Advani SH, Nadkarni JJ. Impaired T lymphocyte function and differential cytokine response pattern in members from cancer families. Nat Immun. 1998;16:146-56.
46. Shevde LA, Joshi NN, Shinde SR, Nadkarni JJ. Studies on functional status of circulating lymphocytes in unaffected members from cancer families. Hum Immunol. 1998;59:373-81.
47. Wang H, Diepgen TL. Is atopy a protective or a risk factor for cancer? A review of epidemiological studies. Allergy. 2005;60:1098-111.
48. Turner MC. Epidemiology: allergy history, IgE, and cancer. Cancer Immunol Immunother. 2012;61:1493-510.
49. Severi G, Baglietto L, Muller DC, English DR, Jenkins MA, Abramson MJ, et al. Asthma, asthma medications, and prostate cancer risk. Cancer Epidemiol Biomarkers Prev. 2010;19:2318-24.
50. Wang H, Rothenbacher D, Low M, Stegmaier C, Brenner H, Diepgen TL. Atopic diseases, immunoglobulin E and risk of cancer of the prostate, breast, lung and colorectum. Int J Cancer. 2006;119:695-701.

51. Talbot-Smith A, Fritschi L, Divitini ML, Mallon DF, Knuiman MW. Allergy, atopy, and cancer: a prospective study of the 1981 Busselton cohort. Am J Epidemiol. 2003;157:606-12.
52. Eriksson NE, Holmen A, Hogstedt B, Mikoczy Z, Hagmar L. A prospective study of cancer incidence in a cohort examined for allergy. Allergy. 1995;50:718-22.
53. McWhorter WP. Allergy and risk of cancer. A prospective study using NHANESI followup data. Cancer. 1988;62:451-5.
54. Mills PK, Beeson WL, Fraser GE, Phillips RL. Allergy and cancer: organ site-specific results from the Adventist Health Study. Am J Epidemiol. 1992;136:287-95.
55. Vojtechova P, Martin RM. The association of atopic diseases with breast, prostate, and colorectal cancers: a meta-analysis. Cancer Causes Control. 2009;20:1091-105.
56. Vesterinen E, Pukkala E, Timonen T, Aromaa A. Cancer incidence among 78,000 asthmatic patients. Int J Epidemiol. 1993;22:976-82.
57. Vena JE, Bona JR, Byers TE, Middleton E, Jr., Swanson MK, Graham S. Allergy-related diseases and cancer: an inverse association. Am J Epidemiol. 1985;122:66-74.
58. Il'yasova D, Colbert LH, Harris TB, Newman AB, Bauer DC, Satterfield S, et al. Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. Cancer Epidemiol Biomarkers Prev. 2005;14:2413-8.
59. Siemes C, Visser LE, Coebergh JW, Splinter TA, Witteman JC, Uitterlinden AG, et al. C-reactive protein levels, variation in the C-reactive protein gene, and cancer risk: the Rotterdam Study. J Clin Oncol. 2006;24:5216-22.
60. Stark JR, Li H, Kraft P, Kurth T, Giovannucci EL, Stampfer MJ, et al. Circulating prediagnostic interleukin-6 and C-reactive protein and prostate cancer incidence and mortality. Int J Cancer. 2009;124:2683-9.
61. Olsen NJ, Olson G, Viselli SM, Gu X, Kovacs WJ. Androgen receptors in thymic epithelium modulate thymus size and thymocyte development. Endocrinology. 2001;142:1278-83.
62. Santagostino A, Garbaccio G, Pistorio A, Bolis V, Camisasca G, Pagliaro P, et al. An Italian national multicenter study for the definition of reference ranges for normal values of peripheral blood lymphocyte subsets in healthy adults. Haematologica. 1999;84:499-504.
63. Jentsch-Ullrich K, Koenigsmann M, Mohren M, Franke A. Lymphocyte subsets' reference ranges in an age- and gender-balanced population of 100 healthy adults--a monocentric German study. Clin Immunol. 2005;116:192-7.
64. Chng WJ, Tan GB, Kuperan P. Establishment of adult peripheral blood lymphocyte subset reference range for an Asian population by single-platform flow cytometry: influence of age, sex, and race and comparison with other published studies. Clin Diagn Lab Immunol. 2004;11:168-73.
65. Bisset LR, Lung TL, Kaelin M, Ludwig E, Dubs RW. Reference values for peripheral blood lymphocyte phenotypes applicable to the healthy adult population in Switzerland. Eur J Haematol. 2004;72:203-12.
66. Reichert T, DeBruyere M, Deneys V, Totterman T, Lydyard P, Yuksel F, et al. Lymphocyte subset reference ranges in adult Caucasians. Clin Immunol Immunopathol. 1991;60:190-208.

Table 3.1 Baseline characteristics by tertile of lymphocyte count in ARIC participants, 1987-1989

	Lymphocyte Count (10^9 cells/L)		
	Tertile 1 (0.09, 1.65)	**Tertile 2** (1.66, 2.14)	**Tertile 3** (2.15, 6.08)
Total, N	3,447	3,453	3,439
Study Site[a], %			
Jackson, MS	23.0	28.1	43.6
Forsyth County, NC	36.2	38.3	26.9
Minneapolis, MN	40.9	33.6	29.5
Male, %	48.7	46.3	40.5
Blacks, %	26.6	32.6	48.7
Age (years), Mean (SD)	54.0 (5.9)	53.9 (5.8)	53.8 (5.7)
Education, %			
<High school	17.4	20.5	27.9
High school or college graduate	40.0	38.8	38.1
Graduate school	42.5	40.7	33.9
Missing, N	8	3	6
BMI (kg/m^2), %			
<18.5	0.7	1.0	0.6
18.5-24.99	38.8	32.7	23.8
25.0 29.99	40.4	39.0	39.9
30.0-34.99	14.6	18.4	22.7
≥35.00	5.5	8.9	13.0
Missing, N	5	2	2
Waist Circumference (cm), Mean (SD)	94.2 (13.1)	96.6 (13.9)	99.1 (14.3)
Missing, N	1	1	1
Cigarette Smoking Status, %			
Never	46.8	41.5	34.5
Former	37.2	33.7	26.5
Current	16.1	24.8	39.0
Missing, N	4	0	4
Pack-Years[b], Mean (SD)	23.8 (21.7)	24.6 (21.9)	25.0 (21.9)
Missing, N	53	64	73
ETS[c] (hours/week), %			
≤1	35.1	30.7	23.4
>1	64.9	69.3	76.6
Missing, N	23	23	16
Alcohol Intake[d], Mean (SD) (g/wk)	110.4 (126.5)	113.7 (140.6)	106.5 (123.0)
Missing, N	13	15	40
CVD[e], %	8.7	9.0	11.7
Hypertension[f], %	32.6	34.2	40.9
Missing, N	1	1	1

Table 3.1 (continued) Baseline characteristics by tertile of lymphocyte count in ARIC participants, 1987-1989

	Lymphocyte Count (10^9 cells/L)		
	Tertile 1 (0.09, 1.65)	**Tertile 2** (1.66, 2.14)	**Tertile 3** (2.15, 6.08)
Diabetes[g], %	8.7	10.8	16.0
Missing, N	5	3	6
Aspirin used[h], %	46.7	47.0	41.4
Missing, N	21	29	32

Abbreviations: BMI, body mass index; SD, standard deviation; ETS, environmental tobacco smoke; CVD, cardiovascular disease. [a]Participants from Washington County, MD were excluded from analyses due to substantial missing WBC subtype count data at this site. [b]Pack-years among ever smokers only. Pack-years was calculated as the average number of cigarettes smoked per day multiplied by the number of years of smoking and divided by 20. [c]ETS defined as the average number of hours per week of close contact with people when they are smoking. [d]Alcohol intake among participants who reported usually having at least one drink per week. [e]CVD defined as having a history of angina pectoris, coronary heart disease, intermittent claudication, or stroke. [f]Hypertension defined as use of any hypertensive medications, systolic blood pressure $\geq$140 mmHg, or diastolic blood pressure $\geq$90 mmHg. [g]Diabetes defined as having a fasting glucose level $\geq$126 mg/dl, non-fasting glucose level $\geq$200 mg/dl, a physician diagnosis of diabetes, or using sugar-lowering medications in two weeks prior to enrollment. [h]Aspirin use in two weeks prior to study enrollment.

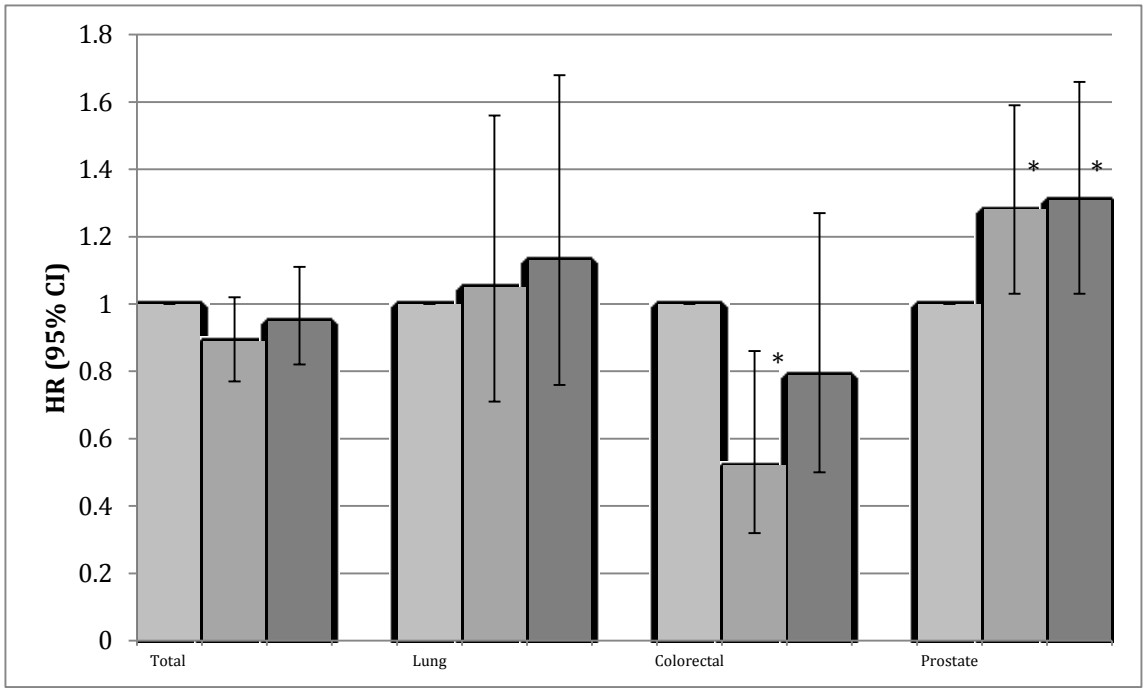

Figure 3.1 Multivariable-adjusted hazard ratios of total and site-specific cancer incidence by tertile of lymphocyte count in men in the ARIC study, 1987-2006. Models adjusted for study site, race, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m^2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (<1, ≥1 hour/week), alcohol intake (continuous), aspirin use in two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. Asterisk indicates p-value≤0.05.

Table 3.2 Multivariable-adjusted hazard ratios for cancer incidence by tertile of lymphocyte count, stratified by race and cigarette smoking status, in men in the ARIC study, 1987-2006

Lymphocyte Count by Tertile	N	Person-Years	Age-Standardized Rate[a]	HR (95% CI)[b]
Overall				
1	431	26120.4	258.1	1.00 (Reference)
2	375	24662.0	243.6	0.89 (0.77, 1.02)
3	347	20469.7	255.7	0.95 (0.82, 1.11)
Excluding Prostate Cancer				
1	275	26120.4	166.2	1.00 (Reference)
2	198	24662.0	126.3	0.68 (0.56, 0.82)
3	204	20469.7	152.2	0.75 (0.62, 0.91)
Whites[c]				
1	208	19464.3	172.8	1.00 (Reference)
2	141	18015.3	126.0	0.65 (0.52, 0.81)
3	142	13144.4	167.1	0.78 (0.62, 0.98)
Blacks[c]				
1	67	6656.1	151.4	1.00 (Reference)
2	57	6646.7	127.0	0.77 (0.54, 1.11)
3	62	7325.3	119.2	0.70 (0.48, 1.01)
Current Smokers[c]				
1	68	3928.6	252.6	1.00 (Reference)
2	75	6408.3	174.8	0.61 (0.44, 0.86)
3	126	8462.2	206.5	0.79 (0.58, 1.07)
Former Smokers[c]				
1	134	13055.8	160.2	1.00 (Reference)
2	83	11234.2	118.4	0.74 (0.56, 0.98)
3	57	7745.1	113.2	0.73 (0.53, 1.01)
Never Smokers[c]				
1	73	9135.9	135.0	1.00 (Reference)
2	40	7019.4	86.7	0.67 (0.45, 1.00)
3	21	4244.3	88.3	0.63 (0.38, 1.05)

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Incidence rate per 1,000 men from 1987 to 2006 standardized to the age and race distribution of the analytic cohort. [b]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke ($\leq$1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [c]Cancer incidence excluding cases of prostate cancer.

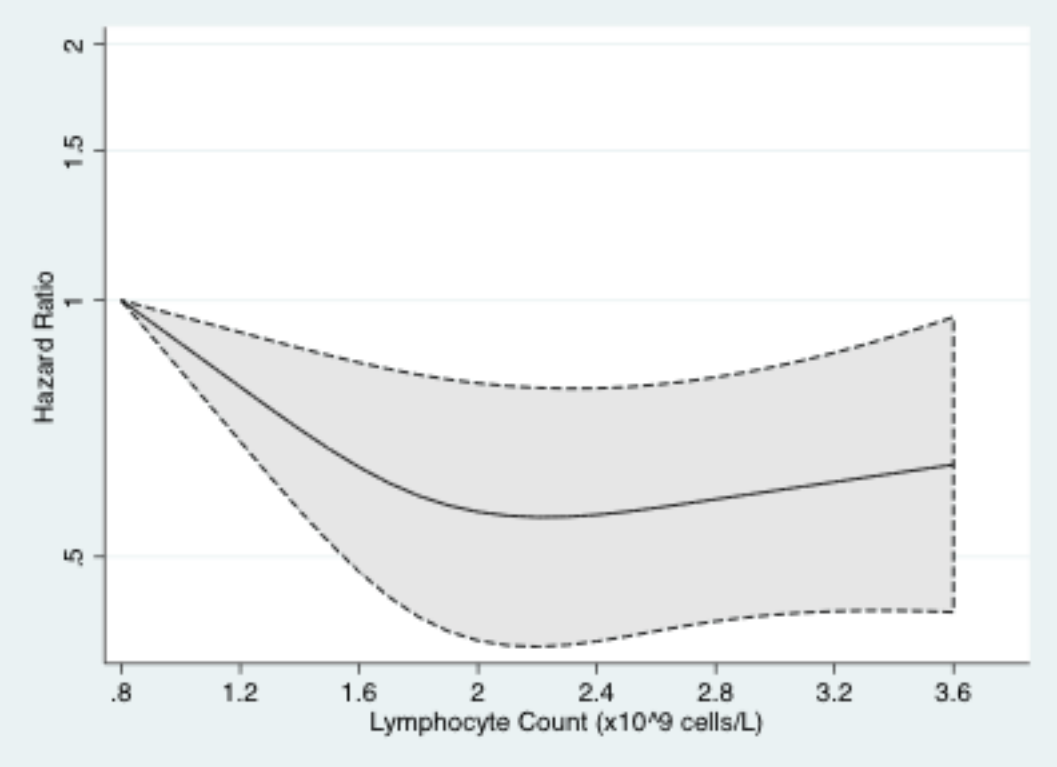

Figure 3.2 Multivariable-adjusted hazard ratios for non-prostate cancer incidence in men using restricted cubic splines with knots at the 10th (1.26 x109 cells/L), 50th (1.84 x109 cells/L), and 90th (2.68 x109 cells/L) percentiles. Gray shading represents 95% confidence intervals. Multivariable models adjusted for study site, race, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m^2), smoking status (never, former, current), pack-years (continuous), and environmental tobacco smoke (<1, ≥1 hour/week).

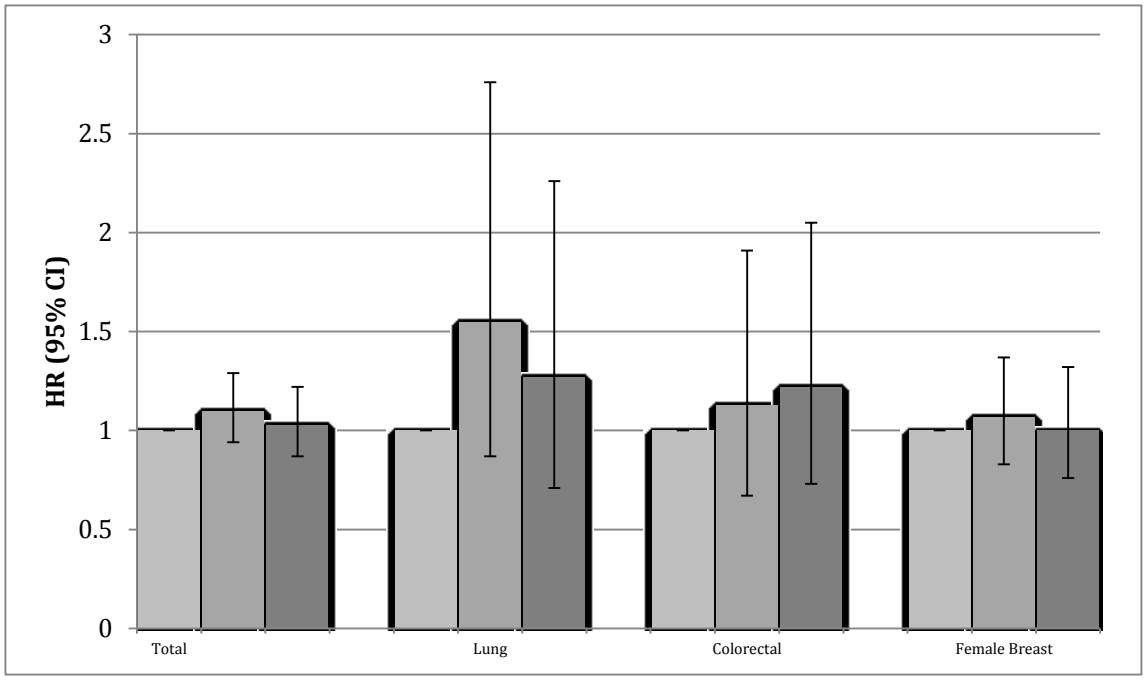

Figure 3.3 Multivariable-adjusted hazard ratios of total and site-specific cancer incidence by tertile of lymphocyte count in women in the ARIC study, 1987-2006. Models adjusted for study site, race, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m^2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (<1, ≥1 hour/week), alcohol intake (continuous), aspirin use in two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, diabetes, menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user).

Table 3.3 Multivariable-adjusted hazard ratios for cancer incidence by tertile of lymphocyte count, stratified by race, menopausal status, and cigarette smoking status, in women in the ARIC study, 1987-2006

Lymphocyte Count by Tertiles	N	Person-Years	Age-Standardized Rate[a]	HR (95% CI) [b]
Overall				
1	279	28413.0	162.8	1.00 (Reference)
2	332	29346.8	184.6	1.10 (0.94, 1.29)
3	351	31416.6	192.0	1.03 (0.87, 1.22)
P-Trend				0.72
Whites				
1	219	21484.6	177.1	1.00 (Reference)
2	234	19250.5	203.3	1.12 (0.93, 1.35)
3	186	14416.4	216.8	1.02 (0.83, 1.25)
P-Trend				0.79
Blacks				
1	60	6928.5	141.4	1.00 (Reference)
2	98	10096.3	156.6	1.04 (0.75, 1.44)
3	165	17000.2	154.9	1.03 (0.76, 1.40)
P-Trend				0.86
Premenopausal				
1	104	10788.3	161.9	1.00 (Reference)
2	90	8778.2	171.5	0.94 (0.68, 1.29)
3	81	7970.4	181.0	1.03 (0.75, 1.42)
P-Trend				0.86
Postmenopausal				
1	175	17551.4	159.3	1.00 (Reference)
2	242	20568.6	190.7	1.23 (1.00, 1.51)
3	270	23446.2	197.6	1.13 (0.91, 1.41)
P-Trend				0.32
Current Smokers				
1	61	4331.2	185.9	1.00 (Reference)
2	100	6187.1	235.2	1.14 (0.83, 1.58)
3	146	11102.0	211.9	0.94 (0.69, 1.28)
P-Trend				0.46
Former Smokers				
1	60	7162.8	140.3	1.00 (Reference)
2	74	7163.2	177.6	1.13 (0.80, 1.60)
3	73	6403.5	182.0	1.17 (0.82, 1.69)
P-Trend				0.39
Never Smokers				
1	157	16914.0	155.0	1.00 (Reference)
2	158	15996.5	167.8	1.04 (0.83, 1.30)
3	132	13872.8	173.8	1.05 (0.82, 1.35)
P-Trend				0.68

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Incidence rate per 1,000 women from 1987 to 2006 standardized to the age and race distribution of the analytic cohort. [b]Models adjusted for study site

Table 3.3 (continued) Multivariable-adjusted hazard ratios for cancer incidence by tertile of lymphocyte count, stratified by race, menopausal status, and cigarette smoking status, in women in the ARIC study, 1987-2006

(Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (≤1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, diabetes, menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user).

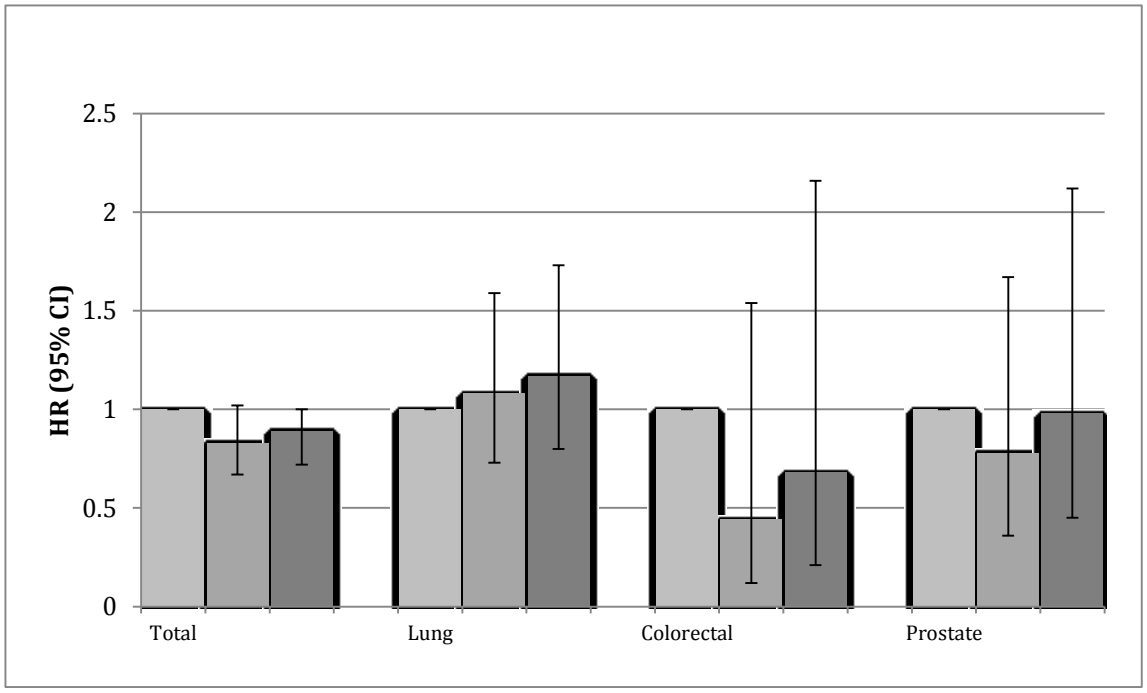

Figure 3.4 Multivariable-adjusted hazard ratios of total and site-specific cancer mortality by tertile of lymphocyte count in men in the ARIC study, 1987-2008. Models adjusted for study site, race, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m^2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (<1, ≥1 hour/week), alcohol intake (continuous), aspirin use in two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. Asterisk indicates p-value≤0.05.

Table 3.4 Multivariable-adjusted hazard ratios for cancer mortality by tertile of lymphocyte count, stratified by race and cigarette smoking status, in men in the ARIC study, 1987-2008

Lymphocyte Count by Tertile	N	Person-Years	Age-Standardized Rate[a]	HR (95% CI)[b]
Overall				
1	192	32337.7	115.0	1.00 (Reference)
2	166	30323.1	108.1	0.83 (0.67, 1.02)
3	181	25245.7	132.1	0.89 (0.72, 1.10)
P-Trend				0.28
Excluding Prostate Cancer				
1	176	32261.7	105.1	1.00 (Reference)
2	154	30261.8	100.5	0.83 (0.66, 1.03)
3	168	25175.8	122.9	0.88 (0.70, 1.11)
P-Trend				0.28
Whites				
1	134	24210.4	111.1	1.00 (Reference)
2	111	22162.9	99.2	0.79 (0.61, 1.03)
3	109	16409.2	130.2	0.86 (0.65, 1.13)
P-Trend				0.25
Blacks				
1	58	8127.3	123.8	1.00 (Reference)
2	55	8160.1	128.2	0.87 (0.60, 1.27)
3	72	8836.5	136.2	0.93 (0.64, 1.34)
P-Trend				0.71
Current Smokers				
1	50	4693.5	191.5	1.00 (Reference)
2	78	7573.2	183.1	0.95 (0.66, 1.36)
3	112	10262.3	183.6	0.96 (0.68, 1.35)
P-Trend				0.84
Former Smokers				
1	94	16173.3	110.5	1.00 (Reference)
2	64	13999.8	93.0	0.81 (0.59, 1.13)
3	54	9562.5	102.9	0.95 (0.67, 1.35)
P-Trend				0.65
Never Smokers				
1	48	11470.9	88.3	1.00 (Reference)
2	24	8750.0	55.5	0.59 (0.36, 0.99)
3	15	5399.7	58.6	0.59 (0.32, 1.09)
P-Trend				0.04

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Incidence rate per 1,000 men from 1987 to 2006 standardized to the age and race distribution of the analytic cohort. [b]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke ($\leq$1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes.

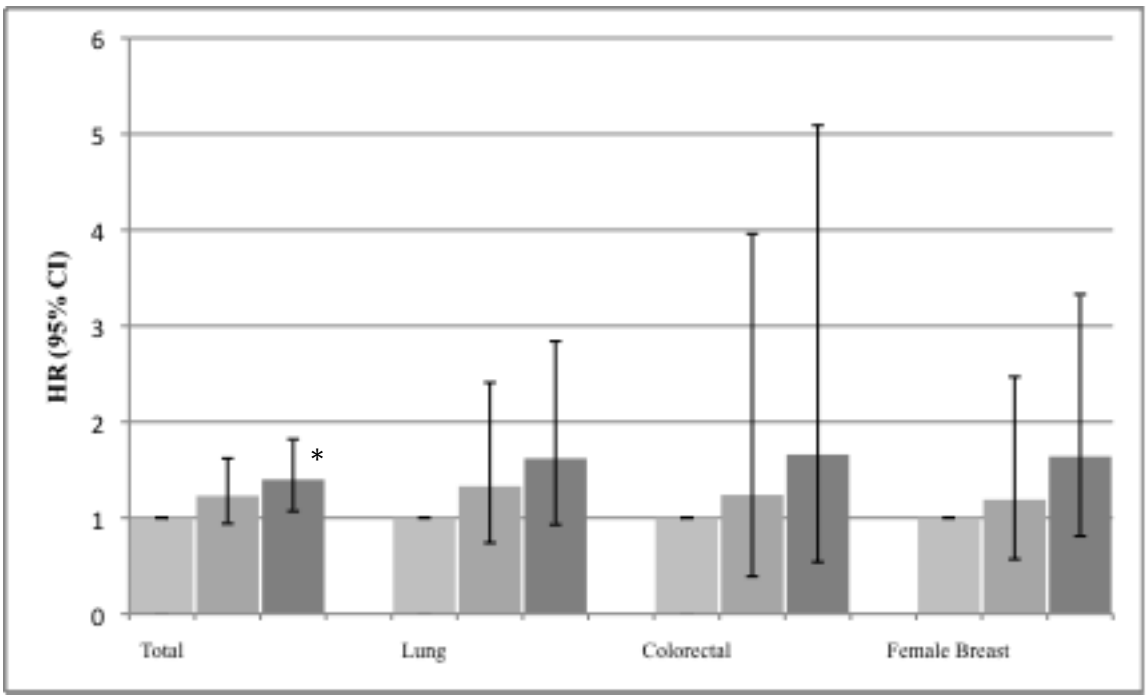

Figure 3.5 Multivariable-adjusted hazard ratios of total and site-specific cancer mortality by tertile of lymphocyte count in women in the ARIC study, 1987-2006. Models adjusted for study site, race, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m^2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (<1, ≥1 hour/week), alcohol intake (continuous), aspirin use in two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, diabetes, menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user). Asterisk indicates p-value ≤0.05.

Table 3.5 Multivariable-adjusted hazard ratios for cancer mortality by tertile of lymphocyte count, stratified by race, menopausal status and cigarette smoking status, in women in the ARIC study, 1987-2008

Lymphocyte Count by Tertiles	N	Person-Years	Age-Standardized Rate[b]	HR (95% CI)[c]
Overall				
1	91	35369.1	52.9	1.00 (Reference)
2	128	36319.5	73.2	1.23 (0.94, 1.62)
3	181	38255.5	95.8	1.40 (1.07, 1.82)
P-Trend				0.02
Whites				
1	72	26782.0	58.1	1.00 (Reference)
2	73	24035.7	63.5	1.02 (0.73, 1.41)
3	95	17823.5	106.8	1.36 (0.98, 1.87)
P-Trend				0.06
Blacks				
1	19	8587.1	45.2	1.00 (Reference)
2	55	12283.8	87.8	1.82 (1.07, 3.09)
3	86	20401.9	79.5	1.65 (1.00, 2.75)
P-Trend				0.14
Premenopausal				
1	25	13540.7	34.9	1.00 (Reference)
2	31	10889.8	58.3	1.33 (0.77, 2.30)
3	40	9744.0	88.4	1.68 (1.00, 2.84)
P-Trend				0.05
Postmenopausal				
1	66	21743.0	60.1	1.00 (Reference)
2	97	25429.6	77.8	1.21 (0.86, 1.70)
3	141	28481.5	100.8	1.39 (1.00, 1.95)
P-Trend				0.05
Current Smokers				
1	27	5295.2	80.2	1.00 (Reference)
2	43	7743.8	95.8	1.15 (0.70, 1.88)
3	103	13326.6	143.1	1.58 (1.02, 2.44)
P-Trend				0.02
Former Smokers				
1	24	8904.5	52.0	1.00 (Reference)
2	34	8755.1	81.2	1.23 (0.72, 2.10)
3	30	7813.8	71.3	1.13 (0.64, 1.99)
P-Trend				0.70
Never Smokers				
1	39	21151.5	41.9	1.00 (Reference)
2	51	19820.6	55.1	1.30 (0.85, 1.98)
3	48	17040.8	58.9	1.31 (0.84, 2.05)
P-Trend				0.13

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Mortality rate per 1,000 women from 1987 to 2008 standardized to the age and race distribution of the analytic cohort. [b]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-29.9,

30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high
Table 3.5 (continued) Multivariable-adjusted hazard ratios for cancer mortality by tertile of lymphocyte count, stratified by race, menopausal status, and cigarette smoking status, in women in the ARIC study, 1987-2006

school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke ($\leq$1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, diabetes, menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user).

Appendix B. Chapter 3 supplemental tables

Table B.1 Baseline characteristics by tertile of lymphocyte count in male and female ARIC participants, 1987-1989

	Lymphocyte Count (x10^9 cells/L)					
	Men (N=4,668)			Women (N=5,671)		
	Tertile 1 (0.09, 1.65)	**Tertile 2** (1.66, 2.14)	**Tertile 3** (2.15, 6.08)	**Tertile 1** (0.09, 1.65)	**Tertile 2** (1.66, 2.14)	**Tertile 3** (2.15, 6.08)
Total, N	1,677	1,598	1,393	1,770	1,855	2,046
Study Site[a], %						
Jackson, MS	23.5	24.4	33.0	22.5	31.2	50.8
Forsyth County, NC	35.1	39.7	30.4	37.2	37.1	24.5
Minneapolis, MN	41.4	35.9	36.5	40.3	31.7	24.7
Black, %	27.0	28.8	37.7	26.2	36.0	56.1
Age (years), Mean (SD)	54.5 (5.8)	54.1 (5.9)	54.2 (5.7)	53.5 (5.8)	53.7 (5.8)	53.6 (5.7)
Education, %						
<High school	18.8	20.6	25.2	16.1	20.4	29.8
High school or college graduate	33.3	34.0	36.3	46.5	42.9	39.4
Graduate school	47.9	45.4	38.5	37.5	36.7	30.9
Missing, N	5	2	2	3	1	4
BMI (kg/m^2), %						
<18.5	0.3	0.6	0.4	1.1	1.4	0.7
18.5-24.99	30.8	27.1	22.4	46.5	37.6	24.8
25.0 29.99	50.1	48.6	48.6	31.2	30.6	34.0
30.0-34.99	15.9	18.3	22.4	13.3	18.5	22.9
≥35.00	2.9	5.3	6.3	7.9	11.9	17.5
Missing, N	2	0	1	3	2	1
Waist Circumference (cm), Mean (SD)	97.5 (10.4)	99.0 (11.5)	100.2 (11.6)	91.1 (14.6)	94.4 (15.3)	98.3 (15.9)
Missing, N	1	0	0	0	1	1

Table B.1 (continued) Baseline characteristics by tertiles of lymphocyte count in male and female ARIC participants, 1987-1989

	Lymphocyte Count (x10^9 cells/L)					
	Men (N=4,668)			Women (N=5,671)		
	Tertile 1 (0.09, 1.65)	**Tertile 2** (1.66, 2.14)	**Tertile 3** (2.15, 6.08)	**Tertile 1** (0.09, 1.65)	**Tertile 2** (1.66, 2.14)	**Tertile 3** (2.15, 6.08)
Cigarette Use, %						
Never	34.0	27.2	19.6	58.9	53.8	44.6
Former	49.9	45.3	36.2	25.1	23.8	19.9
Current	16.2	27.5	44.2	16.0	22.4	35.5
Missing, N	1	0	1	3	0	3
Pack-Years[b], Mean (SD)	27.5 (22.8)	28.4 (22.9)	28.6 (22.7)	19.5 (18.7)	21.7 (20.0)	23.0 (20.3)
Missing, N	31	26	22	25	38	52
ETS[c] (hours/week), %						
≤1	31.4	26.3	20.6	38.5	34.5	25.3
>1	68.6	73.7	79.4	61.5	65.5	74.7
Missing, N	15	9	8	8	14	8
Alcohol Intake[d], Mean (SD) (g/wk)	135.8 (145.4)	140.0 (166.6)	132.6 (142.1)	69.5 (70.9)	72.0 (65.4)	69.6 (75.2)
Missing, N	5	8	17	12	6	20
CVD[e], %	9.6	9.6	13.8	7.8	8.5	10.2
Hypertension[f], %	35.9	35.0	36.1	29.5	33.4	44.1
Missing, N	0	0	1	1	1	0
Diabetes[g], %	10.0	10.4	15.6	7.5	11.1	16.3
Missing, N	3	0	4	2	3	2
Menopausal Status[h]						
Pre/Peri-menopausal	--	--	--	36.7	30.7	29.7
Postmenopausal	--	--	--	63.3	69.3	70.3
HRT Use, %						
Never	--	--	--	10.0	12.1	13.0
Former	--	--	--	37.0	42.2	46.7
Current	--	--	--	37.0	28.8	24.7

Table B.1 (continued) Baseline characteristics by tertiles of lymphocyte count in male and female ARIC participants, 1987-1989

	Lymphocyte Count (x10^9 cells/L)					
	Men (N=4,668)			**Women (N=5,671)**		
	Tertile 1 (0.09, 1.65)	**Tertile 2** (1.66, 2.14)	**Tertile 3** (2.15, 6.08)	**Tertile 1** (0.09, 1.65)	**Tertile 2** (1.66, 2.14)	**Tertile 3** (2.15, 6.08)
Missing, N	--	--	--	33	47	88
Aspirin Used[j], %	42.9	44.2	39.8	50.3	49.3	42.5
Missing, N	13	15	10	8	14	22

Abbreviations: BMI, body mass index; SD, standard deviation; ETS, environmental tobacco smoke; CVD, cardiovascular disease; HRT, hormone replacement therapy. [a]Participants from Washington County, MD were excluded from analyses due to substantial missing WBC subtype count data at this site. [b]Pack-years among ever smokers only. Pack-years was calculated as the average number of cigarettes smoked per day multiplied by the number of years of smoking and divided by 20. [c]ETS defined as the average number of hours per week of close contact with people when they are smoking. [d]Alcohol intake among participants who reported usually having at least one drink per week. [e]CVD defined as having a history of angina pectoris, coronary heart disease, intermittent claudication, or stroke. [f]Hypertension defined as use of any hypertensive medications, systolic blood pressure $\geq$140 mmHg, or diastolic blood pressure $\geq$90 mmHg. [g]Diabetes defined as having a fasting glucose level $\geq$126 mg/dl, non-fasting glucose level $\geq$200 mg/dl, a physician diagnosis of diabetes, or using sugar-lowering medications in two week prior to enrollment. [h]Pre/Peri-menopausal defined as any women who reported having her menstrual period in the two years prior to baseline. [i]Aspirin use in two weeks prior to study enrollment.

Table B.2 Multivariable-adjusted hazard ratios for cancer incidence and mortality by tertile of lymphocyte count accounting for levels of neutrophils, monocytes, eosinophils and basophils in men and women in the ARIC study, 1987-2008

	Men	Women
Lymphocyte Count by Tertile	**HR (95% CI)[a]**	**HR (95% CI)[b]**
Cancer Incidence[c]		
1	1.00 (Reference)	1.00 (Reference)
2	0.69 (0.56, 0.86)	1.22 (1.02, 1.46)
3	0.72 (0.57, 0.91)	1.04 (0.85, 1.26)
Cancer Mortality		
1	1.00 (Reference)	1.00 (Reference)
2	0.84 (0.66, 1.07)	1.48 (1.09, 2.00)
3	0.88 (0.68, 1.14)	1.28 (0.94, 1.75)

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke ($\leq$1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [b]Additional adjustment for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user). [c]Among men, excluding cases of prostate cancer.

Table B.3 Multivariable-adjusted hazard ratios for cancer incidence and mortality by tertile of NLR, stratified by sex, race, menopausal status and cigarette smoking status, in the ARIC study, 1987-2008

	Cancer Incidence	Cancer Mortality
Neutrophil to Lymphocyte Ratio by Tertile[a]	**HR (95% CI)[b]**	**HR (95% CI)[b]**
Overall		
1	1.00 (Reference)	1.00 (Reference)
2	1.07 (0.95, 1.20)	1.17 (0.98, 1.39)
3	1.01 (0.90, 1.14)	1.27 (1.06, 1.52)
P-Trend	0.92	0.009
Males		
1	1.00 (Reference)	1.00 (Reference)
2	1.04 (0.88, 1.22)	1.19 (0.94, 1.52)
3	1.04 (0.88, 1.22)	1.30 (1.02, 1.65)
P-Trend	0.69	0.03
Females[c]		
1	1.00 (Reference)	1.00 (Reference)
2	1.06 (0.90, 1.24)	1.12 (0.87, 1.45)
3	0.90 (0.76, 1.08)	1.13 (0.86, 1.49)
P-Trend	0.26	0.38
Whites		
1	1.00 (Reference)	1.00 (Reference)
2	1.08 (0.93, 1.26)	1.25 (0.97, 1.61)
3	1.03 (0.89, 1.20)	1.36 (1.06, 1.75)
P-Trend	0.91	0.02
Blacks		
1	1.00 (Reference)	1.00 (Reference)
2	1.07 (0.89, 1.28)	1.10 (0.85, 1.43)
3	0.92 (0.74, 1.14)	1.10 (0.82, 1.49)
P-Trend	0.67	0.42
Current Smokers		
1	1.00 (Reference)	1.00 (Reference)
2	0.98 (0.79, 1.21)	1.04 (0.79, 1.36)
3	1.04 (0.84, 1.28)	1.16 (0.89, 1.52)
P-Trend	0.67	0.26
Former Smokers		
1	1.00 (Reference)	1.00 (Reference)
2	1.01 (0.83, 1.24)	1.01 (0.74, 1.39)
3	0.95 (0.77, 1.17)	1.18 (0.86, 1.62)
P-Trend	0.58	0.26
Never Smokers		
1	1.00 (Reference)	1.00 (Reference)
2	1.21 (1.00, 1.45)	1.58 (1.13, 2.21)
3	1.02 (0.83, 1.25)	1.47 (1.03, 2.11)
P-Trend	0.90	0.04

Table B.3 (continued) Multivariable-adjusted hazard ratios for cancer incidence and mortality by tertile NLR, stratified by sex, race, menopausal status and cigarette smoking status, in the ARIC study, 1987-2008

Abbreviations: NLR, neutrophil to lymphocyte ratio; HR, hazard ratio; CI, confidence interval. [a]NLR tertiles defined based on race-specific cut-offs and then combined across the analytic cohort. [b]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke ($\leq$1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [c]Additional adjustment for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user).

Chapter 4. Pre-diagnostic basophil levels and the risk of cancer incidence and mortality in the Atherosclerosis Risk in Communities (ARIC) study

Abstract

Allergies have been associated with a reduced risk of cancer in several studies. The white blood cell (WBC) subtypes, eosinophils and basophils are components of the allergic response. Data from laboratory and observational studies support an anti-tumoral role for eosinophils, however, the role of basopohils in carcinogenesis has not been well studied. In this study, we examined the prospective association between levels of circulating basophils and cancer incidence and mortality in the Atherosclerosis Risk in Communities (ARIC) study. Participants included men and women with total WBC counts within the normal reference range and no history of cancer at baseline (N=10,251). Total WBC and subtype counts were measured at baseline (1987-1989) and cancer incidence and mortality data were ascertained through 2006 and 2008, respectively. Cox proportional hazards models were used to estimate the multivariable-adjusted hazard ratios (HR) and 95% confidence intervals (CI) by the presence ($\geq$1%) or absence (<1%) of basophils in the peripheral blood. During follow-up, a total of 2,138 incident primary cancers and 952 cancer deaths were ascertained. The presence of circulating basophils was associated with a reduced risk of cancer incidence that approached statistical significance (HR: 0.93, 95% CI: 0.85, 1.01) and a significantly reduced risk of cancer mortality (HR: 0.87, 95% CI: 0.76, 1.00). Similar risk estimates were found after mutual adjustment for the other WBC subtypes, including eosinophils, and after excluding persons with basophil counts outside the normal range (>0.19 x10^9 cells/L). In individuals with normal total WBC count, the presence of basophils in the peripheral blood was associated with a reduced risk of subsequent cancer incidence and mortality, independent of the effects of eosinophils. Future studies are warranted to explore to what extent this association is explained by

allergies.

Introduction

In epidemiological studies, a history of allergies has been associated with an overall reduced risk of cancer incidence and mortality, with the most consistent findings for pancreatic cancers and gliomas (1, 2). It is widely hypothesized that the heightened immune response characteristic of allergies may also enhance tumor immunosurveillance, the process in which the immune system identifies and clears premalignant and malignant cells (1, 3, 4). The white blood cell (WBC) subtypes, eosinophils and basophils, are important mediators of the allergic response (5, 6). In in vitro and in vivo studies, eosinophils have also been shown to have anti-tumoral properties (7-9). This is consistent with the findings from a prospective study conducted by co-authors of this paper, in which pre-diagnostic eosinophil count was inversely associated with colorectal cancer incidence (10). Additionally, elevated levels of eosinophils have been associated with improved prognosis of several cancer types, including breast, colorectal and head and neck (11-14).

Basophils, which constitute 0 to 3% of total WBC count, have a unique role in acute and chronic allergic responses, as one of the primary producer of interleukin-4 (IL-4), which, in turn, promotes T helper 2 (Th2) cell differentiation (6, 15-18). However, to date, the role of basophils in carcinogenesis has not been well examined. In one study, cancer patients had lower levels of circulating basophil count compared to non-cancer patients (19). Additionally, in mice models, basophils were found to damage tumor cell receptors, which may hinder tumor growth and progression (20).

In the present study, we examined, for the first time, the association between circulating pre-diagnostic basophil count and cancer incidence and mortality in the Atherosclerosis Risk in Communities (ARIC) cohort, a large, community-based study. We hypothesized that basophil count would be inversely associated with cancer risk, independent of the effects of eosinophil count, indicating a possible role for this cell type in the tumor immunosurveillance response.

Materials and Methods

Study population

This study was conducted in the ARIC study, an ongoing, prospective cohort initiated between 1987 and 1989 to investigate the etiology of atherosclerosis and its sequelae. Men and women (N=15,792), ages 45 to 64 years, were enrolled from four U.S. communities, Forsyth County, NC; Jackson, MS; Washington County, MD; and suburban Minneapolis, MN. Participants were identified using probability sampling in Minneapolis and Washington County, while in Jackson, blacks were exclusively recruited and in Forsyth County blacks were oversampled (21). Participants from Washington County were not included in these analyses as baseline WBC subtype count was missing for more than 85% of participants (10). At enrollment, participants provided a blood sample and reported information on sociodemographic factors, medical history, reproductive history, physical activity, alcohol and tobacco use and other lifestyle behaviors via standardized questionnaires.

The analytic cohort included both men and women that met the following eligibility criteria: (1) not residing in Washington County, MD (N=11,792); (2) no personal history of cancer, excluding cases of non-melanoma skin cancer (N=11,175); (3) white or black race (N=11,140); (4) total WBC count within two standard deviations of the mean in whites and blacks, separately, given the differences in total WBC count by race (22) (N=10,351); (5) not missing baseline basophil count (N=10274); and (6) not missing information on cancer incidence (N=10,114) or cancer mortality (N=10,251).

Exposure ascertainment

Total and differential WBC counts were measured at baseline and three years later at Visit 2. In the main analyses, baseline WBC subtype counts were used. Following venipuncture, samples were stored at 4°C and within 24 hours total WBC count was measured using automated particle counters in local, independent clinical laboratories. Subtypes were measured as a proportion of total WBCs, with a detectable limit of 1%, and absolute WBC subtype counts were calculated by multiplying the subtype proportion by the total WBC count. Based on repeat testing of individuals conducted one to two weeks apart, reliability coefficients for total WBC count were estimated to be greater than 0.96 for each laboratory (23, 24). The distribution of basophil counts in the analytic cohort is presented in Appendix C, Figure C.1.

Outcome ascertainment

Cancer incidence, including date of diagnosis and site of cancer, was ascertained from study initiation through December 31, 2006 (24, 25). Cancer incidence was primarily

identified by linkage to well-established state and/or county cancer registries that have a high completeness (≥90%) of cancer data (24). Hospital surveillance was used to identify cancer cases in Jackson prior to establishment of the Mississippi state cancer registry in 1996 (26), and all additional cancer cases for the other study sites (24, 27). At present, data on stage at diagnosis, cancer subtype, and treatment are not consistently available for all cancers.

Vital status was available through December 31, 2008. Deaths were identified through contact with relatives, physician or designated contact, or through a search of obituaries, funeral and hospital records, death certificates and the National Death Index (NDI). The date and cause of death were confirmed by death certificate for all reported deaths. Cause of death was coded using the Ninth International Statistical Classification of Diseases and Related Health Problems (ICD-9) for deaths through 1998 and ICD-10 for all subsequent deaths. Cause-specific mortality is available for 98% of decedents (28).

Assessment of covariates

Participants reported their highest education attainment, regular alcohol use, and intake of aspirin in the two weeks prior to baseline study visit. They also provided information on current cigarette smoking status and, if applicable, the average number of years of smoking and cigarettes smoked per day. These values were used to calculate pack-years ([cigarettes per day*years smoking]/20 cigarettes per pack) among ever smokers. Exposure to environmental tobacco smoke (ETS) was defined as being in close proximity to smokers for more than 1 hour per week (29, 30). Body mass index (BMI), calculated

as weight (kg) divided by height (m) squared, and waist circumference were collected by trained technicians at baseline study visit. A history of cardiovascular disease (CVD) was defined as having a prior diagnosis of angina pectoris, coronary heart disease, intermittent claudication or stroke. Participants were categorized as having hypertension if they reported use of any hypertensive medications or if they had systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Diabetes was defined as having a fasting glucose level ≥126 mg/dl, a non-fasting glucose level ≥200 mg/dl, a self-reported physician diagnosis of diabetes, or use of blood sugar lowering medications in the two weeks prior to enrollment. History of asthma (ever or never) was based on self-report. Women were categorized as being premenopausal if they had a menstrual cycle within two years of baseline or postmenopausal (31). Women missing information on menopausal status were categorized as being postmenopausal if they were 55 years of age or older. Postmenopausal hormone use was categorized as current, former or never (31).

Statistical analyses

In 50% of the analytic cohort, basophils constituted less than 1% of the total WBC count and, thus, were not quantifiable. Therefore, we created binary categories of basophil count based on the absence (<1%) or presence (≥1%) of basophils in the peripheral blood. Baseline descriptive characteristics were compared by category of basophil level using the chi-square test for categorical variables or ANOVA for continuous variables. Cox proportional hazards models were used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for cancer incidence and mortality by binary categories of basophil level. In analyses of cancer incidence, follow-up time was accrued from age at

baseline blood draw, with the origin defined as age 40 years and staggered late entries for persons over age 40 at baseline, to the first of the following events: (1) age at first primary cancer diagnosis, (2) age at death, or (3) age at end of follow-up (December 31, 2006). In analyses of cancer mortality, individuals were followed from age at baseline blood draw to (1) age at death or (2) age at end of follow-up (December 31, 2008). In models in which time accrued from year at baseline blood draw to (1) year at first primary cancer diagnosis or cancer death, (2) year at other death, or (3) year at end of follow-up, similar HRs were estimated and are not presented here.

Multivariable models included the baseline covariates, race (white, black), study site (Jackson, MS, Forsyth County, Minneapolis, MN), body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m^2), waist circumference (continuous), education attainment (<high school diploma, high school diploma, >high school or college graduate, graduate school), cigarette smoking status (never, former, current), pack-years (continuous), ETS (≤1 hour/week, >1 hour/week), alcohol intake (g/wk), aspirin use in two weeks prior to blood draw (yes/no), and medical history of CVD (yes/no), hypertension (yes/no), and diabetes (yes/no). In women, the additional covariates, menopausal status and HRT use (premenopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user) were included in models. Models mutually adjusting for counts of the other WBC subtypes, neutrophils, lymphocytes, monocytes, and eosinophils, were also conducted. Missing pack-year and waist circumference information was replaced with the median value. Effect modification between basophil count and select covariates known to modulate the

host immune response, including race, sex, cigarette smoking status, BMI, and NSAIDs use, was assessed by introducing a cross-product term in models and using the Wald test to test for statistical significance. Additionally, we evaluated the interaction between basophil count category and eosinophil count tertile to explore a potential synergistic effect between these inflammatory factors. For all models, the proportional hazards assumption was assessed by introducing an interaction term between monocyte count and follow-up time. In all cases, the interaction term was not statistically significant, confirming this assumption. Rates of cancer incidence and cancer mortality were standardized to the age, race, and sex distribution of the analytic cohort.

Cancer site-specific analyses were conducted in models of cancer incidence and cancer mortality for the four most common cancers (i.e., female breast cancer, colorectal cancer, lung cancer, and prostate cancer). Additionally, the following sensitivity analyses were performed: (1) excluding incident cancer cases and deaths within one and five years from baseline blood draw in order to address any bias due to reverse causation, (2) excluding participants who reported a diagnosis of asthma, used aspirin in the two weeks prior to baseline, and ever smokers to address the possibility of residual confounding by these variables; (3) excluding persons with absolute basophil counts outside the normal reference range (>0.19 $x10^9$ cells/L); and (4) using basophil count measured at Visit 2 as the exposure of interest in a subset of the participants with available data (N=6,577); In these analyses, time at risk was initiated at age at Visit 2. Finally, in cancer mortality analyses, sub-distribution hazard ratios were estimated using the Fine and Gray approach

to account for the issue of competing risks (32). All analyses were conducted using STATA version 11.2 (Stata Corporation, College Station, TX, 2012).

Note: The association between eosinophil count and site-specific cancer incidence in this cohort has been previously reported (10). We present the risk of total cancer incidence and mortality by eosionphil count tertile in Appendix C, Tables C.1 and C.2, respectively.

Results

Table 4.1 presents the distribution of baseline characteristics by binary category of basophil level. Among the 50% of the analytic cohort with basophils constituting 1% or greater of the total WBC count (median: 1%, interquartile range (IQR): 1%, 1%), the median basophil count was 0.06 x 10^9 cells/L (IQR: 0.049 to 0.079 x 10^9 cells/L). Participants with $\geq$1% of basophils in the peripheral blood were more likely to be female, black, less educated, have a higher BMI, greater exposure to ETS, and have a medical history of hypertension and diabetes. Among persons with a history of asthma, there was no significant difference in basophil level.

Cancer incidence

Between 1987 and 2006, a total of 163,150.9 person years and 2,138 incident primary cancers were accrued. As presented in Table 4.2, the presence of basophils in the peripheral blood ($\geq$1%) was associated with a 7% (HR: 0.93, 95% CI: 0.85, 1.01) reduced risk of total cancer incidence, although this association did not reach statistical significance. A suggestive inverse association remained after mutual adjustment for the

other WBC subtype counts (HR: 0.94, 95% CI: 0.85, 1.04; Table 4.2), after excluding incident cancer cases that developed within the first five years of baseline, and among non-asthmatics and persons who did not use aspirin in the two weeks prior to blood draw, but no association was found in never smokers (Table 4.2). Forty-six participants had absolute basophil counts exceeding the normal reference range (0 to $0.19x10^9$ cells/L); excluding these individuals did not appreciably alter risk estimates. Additionally, further adjustment for a history of asthma did not significantly alter risk estimates suggesting that basophils do not entirely mediate the effects of asthma.

There was no evidence of a statistical interaction between the presence of basophils and race, sex, cigarette smoking status, BMI, or aspirin use (all p-interaction$\geq$0.3), and the joint effects of high basophil and eosinophil counts were not synergistic (p-interaction term=0.9). Using basophil level measured at Visit 2, a non-significant inverse association was found between basophils and cancer incidence (HR: 0.92, 95% CI: 0.81, 1.05). In cancer incidence site-specific analyses, the presence of basophils was associated with a suggestive reduced risk of lung cancer (HR: 0.94, 95% CI: 0.85, 1.03), but there was no association with colorectal, female breast or prostate cancer incidence (Table 4.3).

Cancer mortality

Between 1987 and 2008, there were 201,099.8 person-years and 952 cancer deaths. Individuals with detectable basophils in the peripheral blood had a 13% (HR: 0.87, 95% CI: 0.76, 1.00) reduced risk of dying of cancer compared to those with no basophils (Table 4.4). Similar risk estimates were found after mutual adjustment for the other WBC

subtypes, including eosinophil count, (HR: 0.87, 95% CI: 0.75, 1.01; Table 4.4). Additionally, there was no evidence of a statistical interaction by race, sex, cigarette smoking status, BMI, aspirin use, or eosinophil count tertile (all p-interaction terms≥0.2). A significant inverse association between basophil level and cancer mortality remained among non-smokers (HR: 0.76, 95% CI: 0.58, 1.00; Table 4.4), non-asthmatics (HR: 0.86, 95% CI: 0.75, 0.99), persons who did not use aspirin in the two weeks prior to blood draw (HR: 0.80, 95% CI: 0.67, 0.95), and persons with absolute basophil counts within the normal reference range (HR: 0.87, 95% CI: 0.76, 1.00). A suggestive, but non-statistically significant inverse association was found after excluding cancer cases within five years of baseline (HR: 0.91, 95% CI: 0.80, 1.05). In multivariable-adjusted models, inclusion of asthma yielded a slightly attenuated but still significant risk estimate, suggesting that asthma does not entirely mediate this association. In the analysis using basophil level measured at Visit 2, a non-significant inverse association was found (HR: 0.86, 95% CI: 0.70, 1.05).

In cancer mortality site-specific analyses, the presence of basophils in the peripheral blood was associated with a significantly reduced risk of lung cancer mortality (HR: 0.78, 95% CI: 0.61, 0.99), but not colorectal, breast or prostate cancer mortality (Table 4.5). Upon further stratification by cigarette smoking use, the significant association with lung cancer mortality was present among current smokers only (HR: 0.74, 95% CI: 0.55, 1.00; Appendix C, Table C.3), while the presence of basophils was associated was a reduced risk of non-lung cancer mortality in never smokers (HR: 0.74, 95% CI: 0.56, 0.99; Appendix C, Table C.3). Lastly, the presence of basophils was not associated with non-

cancer mortality or CVD mortality. In analyses accounting for such competing causes of death, the inverse association with cancer mortality remained.

Discussion

In this prospective cohort study, the presence of basophils in the peripheral blood was associated with a suggestive reduced risk of total cancer incidence and a significantly reduced risk of cancer mortality. These associations were independent of the effects of eosinophils and the other WBC subtypes. Additionally, a significant inverse association with cancer mortality persisted among never smokers, non-asthmatic, and persons with basophil counts within the normal reference range. To our knowledge, this is the first prospective study to examine the association between circulating basophils and cancer incidence and mortality. Our findings lend indirect support to the tumor immuosurveillance concept and suggest that variations of basophil levels within the normal range may reflect the effectiveness of this anti-tumoral mechanism.

Consistent with our findings, Galoppin et al. (19), previously reported lower levels of circulating basophil count in cancer patients compared to healthy persons without cancer. Interestingly, Galoppin et al. (19) also observed a decreased concentration of histamine in the blood of cancer patients, possibly due to this reduced basophil count. This finding suggests that basophils may have an etiological role in carcinogenesis, in part, through the production of histamine, which has the capacity to promote natural killer (NK) cell survival and stimulate NK and T cell activity, cellular components involved in eliminating premalignant and malignant cells (33). Additionally, basophils are a major

producer of the cytokine, IL-4 (16, 17), which may have anti-tumoral properties through the promotion of cytotoxic T lymphocyte activity (34) and the inhibition of tumor cell proliferation (35, 36). Findings from a study conducted in mice, offer an alternate explanation for our findings; in this study, basophils were found to damage tumor cell receptors, which may hinder tumor growth and progression (20).

Eosinophils, another component of total WBC count with a critical role in the allergic response, have been previously found to be inversely associated with colorectal cancer incidence in this cohort (10). A priori, we hypothesized that basophils, which have a non-redundant function in the allergic response (6, 18), may also have an independent or synergistic role in carcinogenesis. In support of this hypothesis, inclusion of eosinophil count and the other WBC subtypes, in multivariable models did not alter the significant association between basophil level and cancer mortality. Of note, we did not find any evidence of a synergistic effect between basophils and eosinophils in models of cancer incidence and mortality.

In the present study, we could not examine if basophils mediate the relationship between allergies and cancer, as data on allergies were not collected. However, by restricting the study population to persons with total WBC counts within the normal reference range, we minimized the number of persons with severe allergic reactions. Indeed, only 0.4% (N=46) of the analytic cohort had basophil counts above the normal reference range, which is commonly indicative of allergic sensitivity (37). Nevertheless, we expect considerably more participants to have milder forms of allergies given that the prevalence

of allergies in the United States has been estimated to be as high as 20% (38). Notably, inclusion of the variable self-reported diagnosis of asthma into models did not have a significant effect on risk estimates, suggesting that our findings are not largely mediated by asthma, a form of allergy. Future studies are necessary to explore the effect of allergies on the association between basophils and cancer and to examine whether allergy induced basophilia is associated with a further reduction of cancer incidence and mortality.

In this study, we also found basophils to be more strongly associated with cancer mortality than cancer incidence. This observation may indicate a specific role for basophils in the development of more aggressive disease. Alternatively, basophil count may modulate treatment responsiveness. In the present study, we were unable to evaluate these possibilities as information on tumor stage and grade at diagnosis and treatment was not widely collected in ARIC.

An additional limitation of the present study is the use of a one-time measure of basophil count. However, in comparisons of baseline basophil count with a one-time repeat measure, collected three years later, we found 63% agreement by binary category, using the kappa statistic. This suggests that the presence of basophils in the peripheral blood is fairly stable over the short-term. Indeed, in sensitivity analyses utilizing basophil level measured at Visit 2 in a subset of the analytic cohort, similar but non-significant findings were estimated for cancer incidence and mortality. Furthermore, we would expect any effects of intra-individual variation to only attenuate our findings. This study also has

several strengths. Few other prospective cohort studies have collected information on both baseline WBC differential counts and subsequent cancer incidence and mortality. Additionally, detailed information was available on a wide-array of socio-demographic characteristics, lifestyle risk factors and medical history.

In conclusion, in individuals with total WBC counts within the normal reference range, the presence of basophils in the peripheral blood was associated with a reduced risk of cancer incidence and mortality. Our findings suggest that basophils may be an independent risk factor for cancer initiation, promotion and progression. Moreover, these findings provide support for the tumor immunosurveillance concept, generally, and may also indicate a specific role for basophils in this process. Future studies are warranted to explore the etiological role of basophils in carcinogenesis and the effect of allergies in this relationship.

Acknowledgments

The authors thank the staff and participants of the ARIC study for their important contributions.

References

1. Turner MC. Epidemiology: allergy history, IgE, and cancer. Cancer Immunol Immunother. 2012;61:1493-510.
2. Turner MC, Chen Y, Krewski D, Ghadirian P, Thun MJ, Calle EE. Cancer mortality among US men and women with asthma and hay fever. Am J Epidemiol. 2005;162:212-21.
3. Turner MC, Chen Y, Krewski D, Ghadirian P. An overview of the association between allergy and cancer. Int J Cancer. 2006;118:3124-32.
4. Wang H, Rothenbacher D, Low M, Stegmaier C, Brenner H, Diepgen TL. Atopic diseases, immunoglobulin E and risk of cancer of the prostate, breast, lung and colorectum. Int J Cancer. 2006;119:695-701.
5. Rothenberg ME, Hogan SP. The eosinophil. Annu Rev Immunol. 2006;24:147-74.
6. Obata K, Mukai K, Tsujimura Y, Ishiwata K, Kawano Y, Minegishi Y, et al. Basophils are essential initiators of a novel type of chronic allergic inflammation. Blood. 2007;110:913-20.
7. Simson L, Ellyard JI, Dent LA, Matthaei KI, Rothenberg ME, Foster PS, et al. Regulation of carcinogenesis by IL-5 and CCL11: a potential role for eosinophils in tumor immune surveillance. J Immunol. 2007;178:4222-9.
8. Roberts RL, Ank BJ, Stiehm ER. Human eosinophils are more toxic than neutrophils in antibody-independent killing. J Allergy Clin Immunol. 1991;87:1105-15.
9. Gleich GJ. Mechanisms of eosinophil-associated inflammation. J Allergy Clin Immunol. 2000;105:651-63.
10. Prizment AE, Anderson KE, Visvanathan K, Folsom AR. Inverse association of eosinophil count with colorectal cancer incidence: atherosclerosis risk in communities study. Cancer Epidemiol Biomarkers Prev. 2011;20:1861-4.
11. Nielsen HJ, Hansen U, Christensen IJ, Reimert CM, Brunner N, Moesgaard F. Independent prognostic value of eosinophil and mast cell infiltration in colorectal cancer tissue. J Pathol. 1999;189:487-95.
12. Ownby HE, Roi LD, Isenberg RR, Brennan MJ. Peripheral lymphocyte and eosinophil counts as indicators of prognosis in primary breast cancer. Cancer. 1983;52:126-30.
13. Goldsmith MM, Belchis DA, Cresson DH, Merritt WD, 3rd, Askin FB. The importance of the eosinophil in head and neck cancer. Otolaryngol Head Neck Surg. 1992;106:27-33.
14. Bethwaite PB, Holloway LJ, Yeong ML, Thornton A. Effect of tumour associated tissue eosinophilia on survival of women with stage IB carcinoma of the uterine cervix. J Clin Pathol. 1993;46:1016-20.
15. Mukai K, Matsuoka K, Taya C, Suzuki H, Yokozeki H, Nishioka K, et al. Basophils play a critical role in the development of IgE-mediated chronic allergic inflammation independently of T cells and mast cells. Immunity. 2005;23:191-202.
16. Schroeder JT, MacGlashan DW, Jr., Lichtenstein LM. Human basophils: mediator release and cytokine production. Adv Immunol. 2001;77:93-122.
17. Voehringer D, Shinkai K, Locksley RM. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. Immunity. 2004;20:267-77.

18. Karasuyama H, Mukai K, Tsujimura Y, Obata K. Newly discovered roles for basophils: a neglected minority gains new respect. Nat Rev Immunol. 2009;9:9-13.
19. Galoppin L, Noirot C, Wastiaux JP, Scheinmann P, Paupe J, Burtin C. Comparison between number of basophils, blood histamine, and histamine release in cancer and noncancer patients. J Allergy Clin Immunol. 1989;84:501-6.
20. Dvorak AM, Galli SJ, Galli AS, Hammond ME, Churchill WH, Jr., Dvorak HF. Tumor-basophil interactions in vitro--a scanning and transmission electron microscopic study. J Immunol. 1979;122:2447-57.
21. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. Am J Epidemiol. 1989;129:687-702.
22. Lim EM, Cembrowski G, Cembrowski M, Clarke G. Race-specific WBC and neutrophil count reference intervals. Int J Lab Hematol. 2010;32:590-7.
23. Nieto FJ, Szklo M, Folsom AR, Rock R, Mercuri M. Leukocyte count correlates in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol. 1992;136:525-37.
24. Ahmed RL, Schmitz KH, Anderson KE, Rosamond WD, Folsom AR. The metabolic syndrome and risk of incident colorectal cancer. Cancer. 2006;107:28-36.
25. Prizment AE, Anderson KE, Visvanathan K, Folsom AR. Association of inflammatory markers with colorectal cancer incidence in the atherosclerosis risk in communities study. Cancer Epidemiol Biomarkers Prev. 2011;20:297-307.
26. Mississippi Cancer Registry Reporting Manual Revised 2011. Manual2011.
27. Prizment AE, Folsom AR, Dreyfus J, Anderson KE, Visvanathan K, Joshu CE, et al. Plasma C-reactive protein, genetic risk score, and risk of common cancers in the Atherosclerosis Risk in Communities study. Cancer Causes Control. 2013;24:2077-87.
28. Rose KM, Eigenbrodt ML, Biga RL, Couper DJ, Light KC, Sharrett AR, et al. Orthostatic hypotension predicts mortality in middle-aged adults: the Atherosclerosis Risk In Communities (ARIC) Study. Circulation. 2006;114:630-6.
29. Howard G, Wagenknecht LE, Burke GL, Diez-Roux A, Evans GW, McGovern P, et al. Cigarette smoking and progression of atherosclerosis: The Atherosclerosis Risk in Communities (ARIC) Study. JAMA. 1998;279:119-24.
30. Kan H, Heiss G, Rose KM, Whitsel EA, Lurmann F, London SJ. Prospective analysis of traffic exposure as a risk factor for incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study. Environ Health Perspect. 2008;116:1463-8.
31. Nabulsi AA, Folsom AR, Szklo M, White A, Higgins M, Heiss G. No association of menopause and hormone replacement therapy with carotid artery intima-media thickness. Atherosclerosis Risk in Communities (ARIC) Study Investigators. Circulation. 1996;94:1857-63.
32. Fine J, Gray R. A proportional Hazards Model for the Subdistribution of Competing Risk. Journal of the American Statistical Association. 1999;94:496-509.
33. Stanciu L. Immunomodulation by histamine. Ann Biol Clin (Paris). 1990;48:623-5.
34. Rogy MA, Beinhauer BG, Reinisch W, Huang L, Pokieser P. Transfer of interleukin-4 and interleukin-10 in patients with severe inflammatory bowel disease of the rectum. Hum Gene Ther. 2000;11:1731-41.

35. Topp MS, Papadimitriou CA, Eitelbach F, Koenigsmann M, Oelmann E, Koehler B, et al. Recombinant human interleukin 4 has antiproliferative activity on human tumor cell lines derived from epithelial and nonepithelial histologies. Cancer Res. 1995;55:2173-6.
36. Topp MS, Koenigsmann M, Mire-Sluis A, Oberberg D, Eitelbach F, von Marschall Z, et al. Recombinant human interleukin-4 inhibits growth of some human lung tumor cell lines in vitro and in vivo. Blood. 1993;82:2837-44.
37. Clinical Guide to Laboratory Tests. 3rd ed. Philadelphia, PA: W.B. Saunders; 1995.
38. Gergen PJ, Turkeltaub PC, Kovar MG. The prevalence of allergic skin test reactivity to eight common aeroallergens in the U.S. population: results from the second National Health and Nutrition Examination Survey. J Allergy Clin Immunol. 1987;80:669-79.

Table 4.1 Baseline characteristics by the absence/presence of basophils in the peripheral blood in ARIC participants, 1987-1989

	Basophils	
	Absence (<1%)	**Presence (≥1%)**
Total, N	5,193	5,081
Study Site[a], %		
Jackson, MS	26.1	36.4
Forsyth County, NC	26.4	41.8
Minneapolis, MN	47.6	21.8
Male, %	46.3	44.0
Blacks, %	30.6	40.7
Age (years), Mean (SD)	53.8 (5.7)	54.0 (5.8)
Education, %		
<High school	18.8	24.8
High school or college graduate	40.7	37.4
Graduate school	40.5	37.8
Missing, N	9	8
BMI (kg/m^2), %		
<18.5	0.5	1.1
18.5-24.99	31.3	32.5
25.0 29.99	40.6	38.9
30.0-34.99	18.6	18.5
≥35.00	9.1	9.0
Missing, N	4	5
Waist Circumference (cm), Mean (SD)	96.5 (13.8)	96.7 (14.0)
Missing, N	2	1
Cigarette Smoking Status, %		
Never	40.6	41.1
Former	33.8	31.2
Current	25.6	27.6
Missing, N	5	3
Pack-Years[b], Mean (SD)	25.0 (21.8)	25.0 (21.9)
Missing, N	80	108
ETS[c] (hours/week), %		
≤1	30.9	28.6
>1	69.1	71.5
Missing, N	28	34
Alcohol Intake[d], Mean (SD) (g/wk)	108.9 (127.2)	112.0 (134.3)
Missing, N	27	32
CVD[e], %	9.8	9.8

Table 4.1 (continued) Baseline characteristics by the absence/presence of basophil count in the peripheral blood in ARIC participants

	Basophils	
	Absence (<1%)	**Presence (≥1%)**
Hypertension[f], %	34.2	37.2
Missing, N	1	2
Diabetes[g], %	11.2	12.3
Missing, N	8	6
Asthma, %	4.6	5.2
Missing, N	4	2
Aspirin used[h], %	45.9	44.4
Missing, N	35	47

Abbreviations: BMI, body mass index; SD, standard deviation; ETS, environmental tobacco smoke; CVD, cardiovascular disease; HRT, hormone replacement therapy. [a]Participants from Washington County, MD were excluded from analyses due to substantial missing WBC subtype count data at this site. [b]Pack-years among ever smokers only. Pack-years was calculated as the average number of cigarettes smoked per day multiplied by the number of years of smoking and divided by 20. [c]ETS defined as the average number of hours per week of close contact with people when they are smoking. [d]Alcohol intake among participants who reported usually having at least one drink per week. [e]CVD defined as having a history of angina pectoris, coronary heart disease, intermittent claudication, or stroke. [f]Hypertension defined as use of any hypertensive medications, systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg. [g]Diabetes defined as having a fasting glucose level ≥126 mg/dl, non-fasting glucose level ≥200 mg/dl, a physician diagnosis of diabetes, or using sugar-lowering medications in two weeks prior to enrollment. [h]Aspirin use in two weeks prior to study enrollment.

Table 4.2 Multivariable-adjusted hazard ratios for cancer incidence by the absence/presence of basophils, stratified by race, sex and cigarette smoking status, in the ARIC study, 1987-2006

	N	Person-Years	Age-Standardized Rate[a]	HR (95% CI)[b]	HR (95% CI)[c]
Overall					
Absence (<1%)	1113	81841.6	218.5	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	1009	80345.0	202.9	0.93 (0.85, 1.01)	0.94 (0.85, 1.04)
Whites					
Absence (<1%)	815	57666.0	218.5	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	618	48861.7	202.9	0.89 (0.79, 0.99)	0.90 (0.79, 1.02)
Blacks					
Absence (<1%)	298	24175.6	194.6	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	391	31483.4	194.5	0.98 (0.84, 1.14)	0.99 (0.84, 1.17)
Males					
Absence (<1%)	608	36813.2	259.4	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	536	34121.1	243.7	0.90 (0.79, 1.02)	0.95 (0.83, 1.09)
Females[d]					
Absence (<1%)	505	45028.3	184.6	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	473	46224.0	169.1	0.94 (0.82, 1.07)	0.91 (0.79, 1.06)
Current Smokers					
Absence (<1%)	351	19608.4	184.6	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	320	21085.8	169.1	0.86 (0.74, 1.01)	0.85 (0.72, 1.02)
Former Smokers					
Absence (<1%)	381	27960.0	266.7	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	328	25088.8	236.0	0.92 (0.79, 1.09)	0.90 (0.76, 1.08)
Never Smokers					
Absence (<1%)	380	34231.9	183.0	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	361	34150.4	175.9	0.98 (0.84, 1.14)	1.02 (0.86, 1.21)

Table 4.2 (continued) Multivariable-adjusted hazard ratios for cancer incidence by the absence/presence of basophils, stratified by race, sex and cigarette smoking status, in the ARIC study, 1987-2006

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Incidence rate per 1,000 individuals from 1987 to 2008 standardized to the age, race and sex distribution of the analytic cohort. [b]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke ($\leq$1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [c]Additionally adjusted for baseline counts of neutrophils, lymphocytes, monocytes and eosinophils. [d]Additionally adjusted for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user.

Table 4.3 Multivariable-adjusted hazard ratios of site-specific cancer incidence by the absence/prescence of basophls in the ARIC study, 1987-2006

	N	Person-Years	HR (95% CI)[a]
Lung			
Absence (<1%)	146	81841.6	1.00 (Reference)
Presence (≥1%)	133	80345.0	0.93 (0.85, 1.03)
Colorectal			
Absence (<1%)	109	81841.6	1.00 (Reference)
Presence (≥1%)	99	80345.0	0.89 (0.67, 1.19)
Breast[b]			
Absence (<1%)	182	45028.3	1.00 (Reference)
Presence (≥1%)	190	46224.0	1.05 (0.85, 1.30)
Prostate[c]			
Absence (<1%)	244	36737.9	1.00 (Reference)
Presence (≥1%)	227	34037.9	0.95 (0.79, 1.15)

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (≤1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [b]Among women only; Additionally adjusted for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user). [c]Among men only.

Table 4.4 Multivariable-adjusted hazard ratios for cancer mortality by absence/presence of basophils, stratified by race, sex and cigarette smoking status, in the ARIC study, 1987-2008

	N	Person-Years	Age-Standardized Rate[a]	HR (95% CI)[b]	HR (95% CI)[c]
Overall					
Absence (<1%)	506	101458.7	100.0	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	441	98438.1	86.4	0.87 (0.76, 1.00)	0.87 (0.75, 1.01)
Whites					
Absence (<1%)	343	72053.0	95.6	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	258	60310.7	85.1	0.88 (0.75, 1.05)	0.89 (0.74, 1.09)
Blacks					
Absence (<1%)	163	29405.6	108.0	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	183	38127.4	88.9	0.84 (0.68, 1.04)	0.84 (0.66, 1.05)
Males					
Absence (<1%)	287	45367.2	113.5	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	249	41807.5	106.0	0.88 (0.73, 1.05)	0.93 (0.75, 1.13)
Females[d]					
Absence (<1%)	219	55821.5	78.6	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	192	56630.6	65.3	0.85 (0.69, 1.04)	0.79 (0.63, 0.99)
Current Smokers					
Absence (<1%)	216	24048.9	159.0	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	194	25192.6	151.2	0.85 (0.70, 1.04)	0.89 (0.71, 1.11)
Former Smokers					
Absence (<1%)	156	34711.0	86.9	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	146	30844.7	86.0	1.01 (0.79, 1.28)	0.99 (0.76, 1.30)
Never Smokers					
Absence (<1%)	133	42638.5	64.7	1.00 (Reference)	1.00 (Reference)
Presence (≥1%)	101	42377.7	41.5	0.76 (0.58, 1.00)	0.67 (0.50, 0.91)

Table 4.4 (continued) Multivariable-adjusted hazard ratios for cancer mortality by absence/presence of basophils, stratified by race, sex and cigarette smoking status, in the ARIC study, 1987-2008

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Mortality rate per 1,000 individuals from 1987 to 2008 standardized to the age, race and sex distribution of the analytic cohort. [b]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (≤1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [c]Additionally adjusted for baseline counts of neutrophils, lymphocytes, monocytes and eosinophils. [d]Additionally adjusted for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user).

Table 4.5 Multivariable-adjusted hazard ratios of site-specific cancer mortality by the presence/absence of basophils in the ARIC study, 1987-2008

	N	Person-Years	HR (95% CI)[a]
Lung			
Absence (<1%)	162	101458.7	1.00 (Reference)
Presence (≥1%)	131	98438.1	0.78 (0.61, 0.99)
Colorectal			
Absence (<1%)	16	101458.7	1.00 (Reference)
Presence (≥1%)	25	98438.1	1.61 (0.84, 3.09)
Breast[b]			
Absence (<1%)	27	55821.5	1.00 (Reference)
Presence (≥1%)	34	56630.6	1.14 (0.68, 1.92)
Prostate[c]			
Absence (<1%)	20	45559.4	1.00 (Reference)
Presence (≥1%)	20	41723.8	1.16 (0.60, 2.24)

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (≤1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [b]Among women only; Additionally adjusted for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user). [c]Among men only.

Appendix C. Chapter 4 supplemental tables and figures

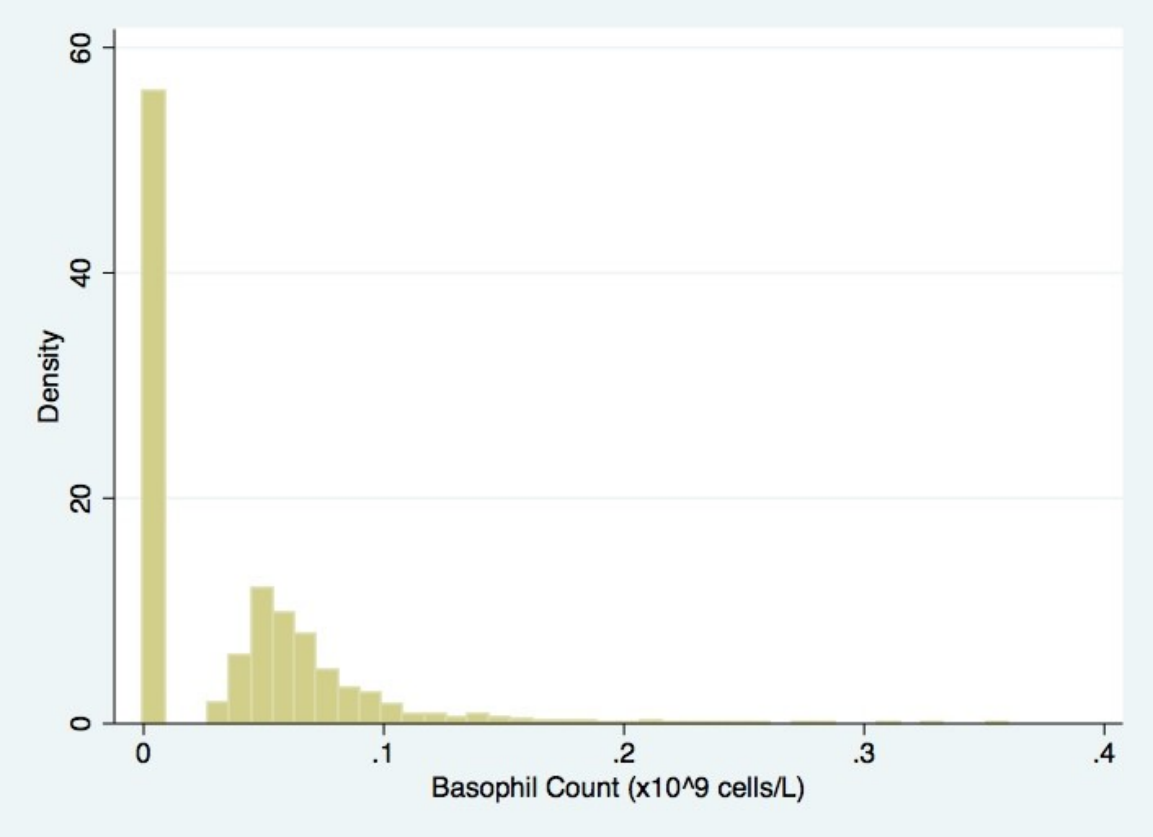

Figure C.1 Distribution of basophil count in the analytic cohort (N=10,251)

Table C.1 Multivariable-adjusted hazard ratio for cancer incidence by tertile of eosinophil count, stratified by race, sex and cigarette smoking status, in ARIC participants, 1987-2006

Eosinophil Count ($x10^9$ cells/L)	N	Person-Years	HR (95% CI)[a]
Overall			
Tertile 1 (<0.07)	747	55223.5	1.00 (Reference)
Tertile 2 (0.07-0.174)	663	54238.0	0.87 (0.78, 0.98)
Tertile 3 (≥0.175)	723	53338.1	0.92 (0.82, 1.03)
P-Trend			0.20
Whites			
Tertile 1 (<0.07)	560	39792.9	1.00 (Reference)
Tertile 2 (0.07-0.174)	401	32339.3	1.01 (0.62, 1.62)
Tertile 3 (≥0.175)	473	34395.9	0.70 (0.43, 1.16)
P-Trend			0.15
Blacks			
Tertile 1 (<0.07)	187	15430.7	1.00 (Reference)
Tertile 2 (0.07-0.174)	262	21898.7	0.82 (0.50, 1.37)
Tertile 3 (≥0.175)	250	18942.2	0.73 (0.42, 1.27)
P-Trend			0.89
Males			
Tertile 1 (<0.07)	390	24080.3	1.00 (Reference)
Tertile 2 (0.07-0.174)	337	21508.4	0.71 (0.42, 1.20)
Tertile 3 (≥0.175)	424	25555.1	0.68 (0.40, 1.14)
P-Trend			0.16
Females			
Tertile 1 (<0.07)	357	31143.3	1.00 (Reference)
Tertile 2 (0.07-0.174)	326	32729.6	1.07 (0.66, 1.71)
Tertile 3 (≥0.175)	299	27783.0	0.75 (0.43, 1.28)
P-Trend			0.29
Current Smokers			
Tertile 1 (<0.07)	192	10508.3	1.00 (Reference)
Tertile 2 (0.07-0.174)	208	13539.1	0.86 (0.69, 1.06)
Tertile 3 (≥0.175)	275	16788.6	0.89 (0.72, 1.09)
P-Trend			0.36
Former Smokers			
Tertile 1 (<0.07)	272	20188.5	1.00 (Reference)
Tertile 2 (0.07-0.174)	207	16490.5	0.85 (0.69, 1.03)
Tertile 3 (≥0.175)	232	16560.1	0.95 (0.78, 1.15)
P-Trend			0.69
Never Smokers			
Tertile 1 (<0.07)	282	24503.6	1.00 (Reference)
Tertile 2 (0.07-0.174)	248	24208.4	0.90 (0.74, 1.08)
Tertile 3 (≥0.175)	216	19951.1	0.92 (0.76, 1.12)
P-Trend			0.43

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), sex, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9,

Table C.1 (continued) Multivariable-adjusted hazard ratio for cancer incidence by tertile of eosinophil count, stratified by race, sex and cigarette smoking status, in ARIC participants, 1987-2008

>35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke ($\leq$1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [b]Among women only; Additionally adjusted for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user).

Table C.2 Multivariable-adjusted hazard ratio for cancer mortality by tertile of eosinophil count, stratified by race, sex and cigarette smoking status, in ARIC participants, 1987-2008

Eosinophil Count ($x10^9$ cells/L)	N	Person-Years	HR (95% CI)[a]
Overall			
Tertile 1 (<0.07)	307	69018.1	1.00 (Reference)
Tertile 2 (0.07-0.174)	314	66358.1	1.00 (0.84, 1.18)
Tertile 3 (≥0.175)	328	65238.3	0.97 (0.82, 1.15)
P-Trend			0.71
Whites			
Tertile 1 (<0.07)	220	50102.9	1.00 (Reference)
Tertile 2 (0.07-0.174)	176	39915.3	0.99 (0.79, 1.24)
Tertile 3 (≥0.175)	205	42358.2	0.94 (0.75, 1.17)
P-Trend			0.55
Blacks			
Tertile 1 (<0.07)	87	18915.2	1.00 (Reference)
Tertile 2 (0.07-0.174)	138	26442.8	1.02 (0.78, 1.34)
Tertile 3 (≥0.175)	123	22970.1	1.02 (0.77, 1.35)
P-Trend			0.91
Males			
Tertile 1 (<0.07)	176	30007.0	1.00 (Reference)
Tertile 2 (0.07-0.174)	165	26333.5	0.95 (0.75, 1.20)
Tertile 3 (≥0.175)	196	31434.3	0.91 (0.72, 1.14)
P-Trend			0.42
Females[b]			
Tertile 1 (<0.07)	131	39011.1	1.00 (Reference)
Tertile 2 (0.07-0.174)	149	40024.6	1.04 (0.81, 1.34)
Tertile 3 (≥0.175)	132	33894.0	1.03 (0.79, 1.34)
P-Trend			0.86
Current Smokers			
Tertile 1 (<0.07)	117	12996.8	1.00 (Reference)
Tertile 2 (0.07-0.174)	128	16297.4	0.86 (0.66, 1.13)
Tertile 3 (≥0.175)	168	20108.9	0.90 (0.69, 1.17)
P-Trend			0.50
Former Smokers			
Tertile 1 (<0.07)	110	25040.5	1.00 (Reference)
Tertile 2 (0.07-0.174)	102	20253.0	1.10 (0.82, 1.50)
Tertile 3 (≥0.175)	89	20488.7	0.96 (0.70,1.31)
P-Trend			0.74
Never Smokers			
Tertile 1 (<0.07)	79	30941.7	1.00 (Reference)
Tertile 2 (0.07-0.174)	84	29807.6	1.12 (0.80, 1.57)
Tertile 3 (≥0.175)	71	24686.4	1.12 (0.79, 1.58)
P-Trend			0.54

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), sex, body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (≤1, >1 hour/week), alcohol intake (continuous), aspirin

Table C.2 (continued) Multivariable-adjusted hazard ratio for cancer mortality by eosinophil count tertile, stratified by race, sex and cigarette smoking status, in ARIC participants, 1987-2008

use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [b]Among women only; Additionally adjusted for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user).

Table C.3 Multivariable-adjusted hazard ratios for non-lung and lung cancer mortality by the absence/presence of basophils, stratified by cigarette smoking status, in the ARIC study, 1987-2008

	Non-Lung Cancer			Lung Cancer		
	N	**Person-Years**	**HR (95% CI)[a]**	**N**	**Person-Years**	**HR (95% CI)[a]**
Current Smokers						
Absence (<1%)	112	24048.9	1.00 (Reference)	104	24048.9	1.00 (Reference)
Presence (≥1%)	108	25192.6	0.96 (0.73, 1.27)	86	25192.6	0.74 (0.55, 1.00)
Former Smokers						
Absence (<1%)	113	34711.0	1.00 (Reference)	43	34711.0	1.00 (Reference)
Presence (≥1%)	111	30844.7	1.07 (0.81, 1.42)	35	30844.7	0.82 (0.52, 1.33)
Never Smokers						
Absence (<1%)	121	42638.5	1.00 (Reference)	12	42638.5	1.00 (Reference)
Presence (≥1%)	92	42377.7	0.74 (0.56, 0.99)	9	42377.7	0.92 (0.37, 2.28)

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (≤1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes.

Chapter 5. No association between pre-diagnostic monocyte count and cancer incidence and mortality

Abstract

Monocytes are an important component of the host inflammatory response. Macrophages, which are derived from circulating monocytes, are one of the predominant immune cells in the tumor microenvironment and have been shown to promote tumor progression in this context. In this study, we examined the relationship between circulating, pre-diagnostic monocyte count and subsequent cancer incidence and mortality in the Atherosclerosis Risk in Communities (ARIC) study. Study participants included men and women with total white blood cell (WBC) counts within the normal reference range and no history of cancer at baseline (N=10,255). Total WBC and subtype counts were measured at baseline (1987-1989) and cancer incidence and mortality data were ascertained through 2006 and 2008, respectively. Cox proportional hazards models were used to estimate the multivariable-adjusted hazard ratios (HR) and 95% confidence intervals (CI) by tertile of monocyte count. During follow-up, 2,142 incident primary cancers and 948 cancer deaths were ascertained. There was no association between monocyte count and cancer incidence. Compared to the lowest tertile, the highest tertile of monocyte count was associated with a slight, non-significant increased risk of total cancer mortality (HR: 1.13, 95% CI: 0.97, 1.33) and a significant increased risk of lung cancer-specific mortality (HR: 1.37, 95% CI: 1.03, 1.85). However, these associations did not persist among never smokers or after mutual adjustment for the other WBC subtypes. Among men and women with total WBC counts within then normal reference range, pre-diagnostic circulating monocyte count does not seem to be an independent risk factor for cancer incidence or mortality.

Introduction

Monocytes, a subtype of total white blood cell (WBC) count, are an important component of the host inflammatory response. From circulation, these cells migrate into the tissue and mature into the long-lived cells, macrophages and dendritic cells. Macrophages and dendritic cells, in turn, are a major source of localized growth factors and cytokines, and have the capacity for phagocytosis and antigen presentation (1, 2). Based on both laboratory and clinical studies, macrophages are also a major component of the tumor microenvironment, with well-established roles in the promotion of tumor growth and metastasis (3-5). In cancer patients, a higher count of macrophages in the tumor infiltrate has been associated with poorer prognosis of several cancer types, including bladder (6), breast (7, 8), and thyroid cancers (9). Elevated circulating levels of monocytes at cancer diagnosis have also been associated with poorer survival of cancers, including lymphoma, melanoma, and cervical, liver, and oral cancers (10-17).

A role for macrophages in tumor initiation and promotion is also hypothesized. In this context macrophages may exert pro-tumoral effects, indirectly, as a component of the chronic inflammatory response, or directly, via the induction of genetic damage or instability, effects on matrix remodeling, and the production of growth factors (18-22). However, the relationship between pre-diagnostic monocyte or macrophage count and cancer incidence and mortality has not been well studied. In a small study of 669 Danish adults, elevated monocyte count was associated with an increased risk of overall cancer incidence in multivariable-adjusted models (23). Notably, however, this study did not examine the association with site-specific cancer incidence and the risk of cancer

mortality was not assessed. In another study of nearly 10,000 elderly Japanese men and women, higher pre-diagnostic levels of monocyte count were associated with increased risk of cancer mortality, based on an unadjusted comparison of survival curves (24).

Given the limited prospective data, we investigated the association between pre-diagnostic monocyte count and cancer incidence and mortality among healthy men and women in the Atherosclerosis Risk in Communities (ARIC) cohort, a large, prospective, community-based study. In this study, we used circulating monocyte count as a surrogate of macrophage levels in the tissue. Based on the pro-tumoral role of macrophages in the tumor microenvironment, we hypothesized that higher levels of pre-diagnostic monocyte count would be associated with an increased risk of cancer incidence and mortality.

Materials and Methods

Study population

This study was conducted in the ARIC study, an ongoing, prospective cohort initiated between 1987 and 1989 to investigate the etiology of atherosclerosis and its sequelae. Men and women (N=15,792), ages 45 to 64 years, were enrolled from four U.S. communities, Forsyth County, NC; Jackson, MS; Washington County, MD; and suburban Minneapolis, MN. Participants were identified using probability sampling in Minneapolis and Washington County, while in Jackson, blacks were exclusively recruited and in Forsyth County blacks were oversampled (25). Participants from Washington County were not included in these analyses as baseline WBC subtype count was missing for more than 85% of participants (26). At enrollment, participants provided a blood sample and

reported information on sociodemographic factors, medical history, reproductive history, physical activity, alcohol and tobacco use and other lifestyle behaviors via standardized questionnaires.

The analytic cohort included men and women that met the following eligibility criteria: (1) not residing in Washington County, MD (N=11,792); (2) no personal history of cancer, excluding cases of non-melanoma skin cancer (N=11,175); (3) white or black race (N=11,140); (4) total WBC count within two standard deviations of the mean in whites and blacks, separately, given the differences in absolute total WBC count by race (27) (N=10,351); (5) not missing baseline monocyte count (N=10,278); and (6) not missing information on cancer incidence (N=10,116) or mortality (N=10,255).

Exposure ascertainment

Total and differential WBC counts were measured at baseline and three years later at Visit 2. In the main analyses, we used baseline WBC subtype counts. Following venipuncture, samples were stored at 4°C and within 24 hours total WBC count was measured using automated particle counters in local, independent clinical laboratories. Subtypes were measured as a proportion of total WBCs and counts were calculated by multiplying the subtype proportion by the total WBC count. Based on repeat testing of individuals conducted one to two weeks apart, reliability coefficients for total WBC count were estimated to be greater than 0.96 for each laboratory (28, 29).

Outcome ascertainment

The incidence of a first primary cancer, including date of diagnosis and site of cancer, was ascertained from study initiation through December 31, 2006 (29, 30). Cancer incidence was primarily identified by linkage to well-established state and/or county cancer registries that have a high completeness (≥90%) of cancer data (29). Hospital surveillance was used to identify cancer cases in Jackson prior to establishment of the Mississippi state cancer registry in 1996 (31), and all additional cancer cases for the other study sites (29, 32). At present, data on stage at diagnosis, cancer subtype and treatment are not consistently available for all cancers.

Vital status was available through December 31, 2008. Deaths were identified through contact with relatives, physician or designated contact, or through a search of obituaries, funeral and hospital records, death certificates and the National Death Index (NDI). The date and cause of death were confirmed by death certificate for all reported deaths. Cause of death was coded using the Ninth International Statistical Classification of Diseases and Related Health Problems (ICD-9) for deaths through 1998 and ICD-10 for all subsequent deaths. Cause-specific mortality is available for 98% of decedents.

Assessment of covariates

Participants reported their highest education attainment, regular alcohol use, and intake of aspirin in the two weeks prior to baseline study visit. They also provided information on current cigarette smoking status and, if applicable, the average number of years of smoking and cigarettes smoked per day. These values were used to calculate pack-years ([cigarettes per day*years smoking]/20 cigarettes per pack) among ever smokers.

Exposure to environmental tobacco smoke (ETS) was defined as being in close proximity to smokers for more than 1 hour per week (33, 34). Body mass index (BMI), calculated as weight (kg)/[height(m)]^2, and waist circumference were collected by trained technicians at baseline study visit. A history of cardiovascular disease (CVD) was defined as having a prior diagnosis of angina pectoris, coronary heart disease, intermittent claudication or stroke. Participants were categorized as having hypertension if they reported use of any hypertensive medications or if they had systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Diabetes was defined as having a fasting glucose level ≥126 mg/dl, a non-fasting glucose level ≥200 mg/dl, a self-reported physician diagnosis of diabetes, or use of blood sugar lowering medications in the two weeks prior to enrollment. Women were categorized as being premenopausal if they had a menstrual cycle within two years of baseline or postmenopausal (35). Women missing information on menopausal status were categorized as being postmenopausal if they were 55 years of age or older. Postmenopausal hormone use was categorized as current, former or never (35).

Statistical analyses

Baseline descriptive characteristics were compared by tertile of monocyte count using the chi-square test for categorical variables or ANOVA for continuous variables. Cox proportional hazards models were used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for cancer incidence and mortality by tertile and continuous values of monocyte count. When modeling monocyte count continuously (per $0.1x10^9$ cells/L), the top and bottom 1% of values were excluded to account for the

potential effects of outliers. Continuous and categorical modeling of monocyte count was compared using the Akaike information criterion. Similar HRs were estimated using continuous values of monocyte count and are not presented in this chapter. Tests for linear trend across categories of monocyte count were calculated by introducing the median value of each tertile as continuous variable into models. In analyses of cancer incidence, follow-up time was accrued from age at baseline blood draw, with the origin defined as age 40 years and staggered late entries for persons over age 40 at baseline, to the first of the following events: (1) age at first primary cancer diagnosis, (2) age at death, or (3) age at end of follow-up (December 31, 2006). In analyses of cancer mortality, individuals were followed from age at baseline blood draw to (1) age at death or (2) age at end of follow-up (December 31, 2008). In models in which time accrued from year at baseline blood draw to (1) year at first primary cancer diagnosis or cancer death, (2) year at other death, or (3) year at end of follow-up, similar HRs were estimated and are not presented here.

Multivariable models included the baseline covariates, race (white, black), study site (Jackson, MS, Forsyth County, Minneapolis, MN), BMI (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, $\geq$35.0 kg/m^2), waist circumference (continuous), education attainment (<high school diploma, high school diploma, >high school or college graduate, graduate school), cigarette smoking status (never, former, current), pack-years (continuous), ETS ($\leq$1 hour/week, >1 hour/week), alcohol intake (g/wk), aspirin use in two weeks prior to blood draw (yes/no), and medical history of CVD (yes/no), hypertension (yes/no), and diabetes (yes/no). In women, the additional covariates, menopausal status and HRT use

(premenopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user) were included in models. Additionally, mutual adjustment by counts of the other WBC subtypes, neutrophils, lymphocytes, eosinophils and basophils was also conducted. Missing pack-year and waist circumference information was replaced with the median value. Effect modification between monocyte count and select covariates known to modulate immune response, including race, sex, cigarette smoking status, BMI and recent aspirin use, was assessed by introducing a cross-product term in models and using the Wald test to test for statistical significance. For all models, the proportional hazards assumption was assessed by introducing an interaction term between monocyte count and follow-up time. In all cases, the interaction term was not statistically significant, confirming this assumption. Rates of cancer incidence and cancer mortality were standardized to the age, race, and sex distribution of the analytic cohort.

Cancer site-specific analyses were conducted in models of cancer incidence and cancer mortality for the four most common cancers (i.e., female breast cancer, colorectal cancer, lung cancer, and prostate cancer). Additionally, the following sensitivity analyses were performed: (1) excluding incident cancer cases and deaths within one and five years from baseline blood draw in order to address any bias due to reverse causation; (2) excluding individuals with monocyte counts outside the clinical reference range, 0.2 to 0.95 x 10^9 cells/L, to more rigorously restrict analyses to individuals with no underlying acute or chronic inflammatory conditions; (3) among never smokers and persons who did not report recent aspirin use to account for the possibility of residual confounding by these

variables; and (4) using monocyte count measured at Visit 2 as the exposure of interest among a subset of the participants with available data (N=6,547); In these analyses, time at risk was initiated at age at Visit 2. All analyses were conducted using STATA version 11.2 (Stata Corporation, College Station, TX, 2012).

Results

In the analytic cohort, the median monocyte count was 0.33 x 10^9 cells/L (interquartile range: 0.21, 0.44). Table 5.1 shows the distribution of baseline characteristics by tertile of monocyte count. Monocyte count varied by study site. Individuals with high monocyte count were more likely to be male, white, current cigarette smokers, and to report increased pack-years, exposure to ETS, and aspirin use. Participants with high monocyte count were also more likely to have a larger waist circumference, although there was no difference in BMI, and to have a medical history of CVD and diabetes, compared to participants with low monocyte count.

Cancer incidence

Between 1987 and 2006, 2,142 incident primary cancers were ascertained during a total of 163,160 person-years. Table 5.2 presents the multivariable-adjusted HR of cancer incidence by tertile of monocyte count. There was no association between monocyte count tertile and cancer risk, overall, or in analyses stratified by race, sex or cigarette use (all p-interaction terms $\geq$0.2). There was also no evidence of effect modification by BMI and recent aspirin use. Similar estimates were found after mutual adjustment for the other

WBC subtypes, among persons with monocyte count within the normal reference range, and after using monocyte count measured at Visit 2.

Cancer mortality

Between 1987 and 2008, 948 cancer deaths and 201,209.3 person-years accrued. In the overall study population, individuals in the highest tertile of monocyte count had a slight but not statistically significant increased risk of cancer mortality (HR: 1.13, 95% CI: 0.97, 1.33), compared to the lowest tertile (Table 5.3). However, risks did not increase linearly by tertile of monocyte count (p-trend=0.07). Similar estimates were found after restricting the analysis to persons with monocyte counts within the normal reference range (0.2 to 0.95 $x10^9$ cells/L) and in models using monocyte count measured at Visit 2. However, the positive association between monocyte count and cancer mortality did not persist after excluding cancer deaths within the first several years of follow-up. In stratified analyses, this association was stronger in whites (HR: 1.21, 95% CI: 0.98, 1.48, p-trend=0.03) than blacks (HR: 1.01, 95% CI: 0.77, 1.31, p-trend=0.92) and in women (HR: 1.29, 95% CI: 1.02, 1.64, p-trend=0.03) compared to men (HR: 1.00, 95% CI: 0.81, 1.24, p-trend=0.80), but there was no significant effect modification by race or sex (all p-interaction terms $\geq$0.1). There was no association between monocyte count tertile and cancer mortality in strata of cigarette smoking status (Table 5.3) and aspirin use. There was also no evidence of a statistical interaction between monocyte count tertile and cigarette smoking status, BMI or recent aspirin use (all p-interaction terms $\geq$0.2).

In fully adjusted models, including mutual adjustment of the other WBC subtypes, there was no association between the highest tertile of monocyte count and cancer mortality, overall, or by sex, race or cigarette smoking status (Table 5.3). Rather, in this fully adjusted model, there was a non-significant reduced risk of total cancer mortality in the middle tertile of monocyte count compared to the lowest tertile (HR: 0.87, 95% CI: 0.71, 1.05).

Table 5.4 presents the results for cancer incidence and mortality site-specific analyses. There was no association between monocyte count tertile and lung, colorectal, prostate, or female breast cancer incidence. The highest tertile of monocyte count was associated with a 1.37-fold (HR: 1.37, 95% CI: 1.03, 1.85, p-trend=0.01) increased risk of lung cancer mortality, overall (Table 5.4) and among current smokers only. An increased risk of lung cancer mortality was found after mutual adjustment for the other WBC subtypes, however this estimate was no longer statistically significant. No associations were found for colorectal, prostate or female breast cancer mortality.

Discussion

In this large prospective study, among men and women without clinically evident inflammation (i.e., total WBC counts within the normal reference range), there was no association between monocyte count and cancer incidence. High levels of pre-diagnostic monocyte count were associated with a non-significant increased risk of total cancer mortality and a significant increased risk of lung cancer mortality, however these associations did not persist after mutual adjustment for the other WBC subtypes. Given

these results, circulating levels of pre-diagnostic monocyte count do not seem to be an independent risk marker for cancer incidence or mortality.

Only one previous study has examined the association between pre-diagnostic monocyte count and cancer incidence. Contrary to our finding of a null association, in a study of 669 Danish adults, conducted by Sajadieh et al (23), high monocyte count (>0.60 $x10^9$ cells/L) was associated with a two-fold (HR: 2.00, 95% CI: 1.10, 3.70) increased risk of overall cancer incidence in multivariable-adjusted models. There are several possible explanations for this discrepancy. First, the prior study did not adjust for total WBC or WBC subtype counts, which we found, in analyses of cancer mortality, to attenuate the association with monocyte count. Second, the significant positive association reported by Sajadieh et al may be due to the inclusion of individuals with monocyte counts above the normal reference range. Specifically, in that study, the upper limit of monocyte count was 3.9 $x10^9$ cells/L as compared to the upper limit in the present study, 1.8 $x10^9$ cells/L, and the upper limit of the normal reference range, 0.95 $x10^9$ cells/L. Third, a limitation of the Sajadieh et al study is the lack of detailed information on pack-years and ETS. Based on our study, cigarette smoking is a potentially strong confounder of the association between monocyte count and cancer incidence and mortality. Thus, we cannot exclude the possibility that the increased risk of cancer incidence reported by Sajadieh et al may be explained, in part, by residual confounding by cigarette smoke exposure. Moreover, this bias may be particularly significant given the high prevalence of current smokers at baseline in this cohort (46%).

In the present study, we observed a positive, but not statistically significant, association between monocyte count and total cancer mortality. However, this association was no longer present after mutual adjustment for the other WBC subtypes, suggesting that monocytes may be in the causal pathway between other WBC subtypes and tumor development and progression. This possible explanation is consistent with the current understanding of macrophage activation, in which lymphocytes, neutrophils and basophils are involved in the polarization of macrophages into primarily pro-tumoral or anti-tumoral cells (36).

Based on in vitro and in vivo studies, tumor cells recruit macrophages, which are derived from circulating monocytes, to the tumor and co-opt their functions to promote tumor cell motility and proliferation, tissue remodeling, and angiogenesis (22, 37). Macrophages are also hypothesized to be factors in tumor initiation and promotion, directly, via the production of reactive oxygen species, reactive nitrogen intermediates and growth factors, or indirectly, as a component of the inflammatory response (19-21, 38). Our null findings, however, suggest that pre-diagnostic monocytes may not be a relevant marker of inflammation in the context of tumor development and progression, nor of localized macrophage carcinogenic activity, among adults with no underlying acute immune condition.

Alternatively, the lack of an association in this study may be due to the characterization of circulating monocytes as a single entity. This may not be a biologically meaningful measure given that monocytes consist of three main subtypes and both monocytes and

macrophages have been shown to display impressive plasticity depending, in part, on the inflammatory microenvironment (36, 39). In particular, both monocytes and activated macrophages may be reversibly polarized to the primarily anti-tumoral M1 phenotype or the pro-tumoral M2 phenotype (39). Thus, future studies measuring monocyte subsets as well as other downstream inflammatory products of the monocyte-mediated inflammatory response may be warranted.

Intra-individual variation of monocyte count over time in healthy adults may also account for our null findings. To our knowledge, the intra-individual variation of monocyte count over the short- and long-terms has not been previously investigated. However, in the present study, among a subset of the analytic cohort with a one-time repeat measure of monocyte count from Visit 2, monocyte counts were only weakly correlated when compared continuously (Spearman rho=0.33) and by tertiles (Kappa=0.17). Such large intra-individual variations may have attenuated any true associations.

In addition to potential residual confounding by cigarette smoking and the significant variation of circulating monocyte count over time, another limitation of this study is the absence of information on tumor stage, grade and treatment. Without this information, we were unable to evaluate whether monocyte count has any specific effects on tumor aggressiveness or treatment effectiveness.

Our study also has several strengths. The ARIC study is a prospective, population-based cohort with detailed information collected on a wide-array of socio-demographic

characteristics, lifestyle risk factors and medical history as well as over 20 years of follow-up for cancer incidence and mortality. Additionally, this is one of a few, large cohort studies to have measured baseline WBC subtype counts and reliably ascertained cancer outcomes over time.

In conclusion, among healthy men and women with total WBC counts within the normal reference range, pre-diagnostic levels of monocytes were not an independent risk factor for cancer incidence or mortality. Our findings do not preclude the possibility that more pronounced difference in monocyte count, such as abnormally elevated levels, may be informative for cancer risk. Additionally, levels of macrophages in benign tissue may be a risk marker for subsequent cancer incidence and mortality. Indeed, macrophages are longer lived cells than monocytes and are a major source of localized cytokines and growth factors (2). Future studies evaluating the association between pre-diagnostic macrophage level and cancer incidence and mortality may clarify the etiological role of these cells in the early stages of carcinogenesis.

Acknowledgments

The authors thank the staff and participants of the ARIC study for their important contributions.

References

1. Longo D, Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J. Harrison's Principles of Internal Medicine. 18 ed: McGraw-Hill; 2011.
2. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420:860-7.
3. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454:436-44.
4. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol. 2002;23:549-55.
5. Mantovani A, Sozzani S, Locati M, Schioppa T, Saccani A, Allavena P, et al. Infiltration of tumours by macrophages and dendritic cells: tumour-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Novartis Found Symp. 2004;256:137-45; discussion 46-8, 259-69.
6. Hanada T, Nakagawa M, Emoto A, Nomura T, Nasu N, Nomura Y. Prognostic value of tumor-associated macrophage count in human bladder cancer. Int J Urol. 2000;7:263-9.
7. Tsutsui S, Yasuda K, Suzuki K, Tahara K, Higashi H, Era S. Macrophage infiltration and its prognostic implications in breast cancer: the relationship with VEGF expression and microvessel density. Oncol Rep. 2005;14:425-31.
8. Leek RD, Lewis CE, Whitehouse R, Greenall M, Clarke J, Harris AL. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. Cancer Res. 1996;56:4625-9.
9. Ryder M, Ghossein RA, Ricarte-Filho JC, Knauf JA, Fagin JA. Increased density of tumor-associated macrophages is associated with decreased survival in advanced thyroid cancer. Endocr Relat Cancer. 2008;15:1069-74.
10. Lee YY, Choi CH, Sung CO, Do IG, Huh S, Song T, et al. Prognostic value of pre-treatment circulating monocyte count in patients with cervical cancer: comparison with SCC-Ag level. Gynecol Oncol. 2012;124:92-7.
11. Lee SD, Kim SH, Kim YK, Lee SA, Park SJ. Prognostic Significance of Preoperative Peripheral Blood Monocyte Ratio in Patients with Hepatocellular Carcinoma. World J Surg. 2014.
12. Sasaki A, Kai S, Endo Y, Iwaki K, Uchida H, Tominaga M, et al. Prognostic value of preoperative peripheral blood monocyte count in patients with colorectal liver metastasis after liver resection. J Gastrointest Surg. 2007;11:596-602.
13. Sasaki A, Iwashita Y, Shibata K, Matsumoto T, Ohta M, Kitano S. Prognostic value of preoperative peripheral blood monocyte count in patients with hepatocellular carcinoma. Surgery. 2006;139:755-64.
14. Tsai YD, Wang CP, Chen CY, Lin LW, Hwang TZ, Lu LF, et al. Pretreatment circulating monocyte count associated with poor prognosis in patients with oral cavity cancer. Head Neck. 2013.
15. Wilcox RA, Ristow K, Habermann TM, Inwards DJ, Micallef IN, Johnston PB, et al. The absolute monocyte count is associated with overall survival in patients newly diagnosed with follicular lymphoma. Leuk Lymphoma. 2012;53:575-80.

16. Bari A, Tadmor T, Sacchi S, Marcheselli L, Liardo EV, Pozzi S, et al. Monocytosis has adverse prognostic significance and impacts survival in patients with T-cell lymphomas. Leuk Res. 2013;37:619-23.
17. Rochet NM, Kottschade LA, Grotz TE, Porrata LF, Markovic SN. The Prognostic Role of the Preoperative Absolute Lymphocyte Count and Absolute Monocyte Count in Patients With Resected Advanced Melanoma. Am J Clin Oncol. 2013.
18. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell. 2005;7:211-7.
19. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell. 2010;141:39-51.
20. Meira LB, Bugni JM, Green SL, Lee CW, Pang B, Borenshtein D, et al. DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice. J Clin Invest. 2008;118:2516-25.
21. Pang B, Zhou X, Yu H, Dong M, Taghizadeh K, Wishnok JS, et al. Lipid peroxidation dominates the chemistry of DNA adduct formation in a mouse model of inflammation. Carcinogenesis. 2007;28:1807-13.
22. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer. 2004;4:71-8.
23. Sajadieh A, Mouridsen MR, Selmer C, Intzilakis T, Nielsen OW, Haugaard SB. Monocyte number associated with incident cancer and mortality in middle-aged and elderly community-dwelling Danes. Eur J Cancer. 2011;47:2015-22.
24. Kim KI, Lee J, Heo NJ, Kim S, Chin HJ, Na KY, et al. Differential white blood cell count and all-cause mortality in the Korean elderly. Exp Gerontol. 2013;48:103-8.
25. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. Am J Epidemiol. 1989;129:687-702.
26. Prizment AE, Anderson KE, Visvanathan K, Folsom AR. Inverse association of eosinophil count with colorectal cancer incidence: atherosclerosis risk in communities study. Cancer Epidemiol Biomarkers Prev. 2011;20:1861-4.
27. Lim EM, Cembrowski G, Cembrowski M, Clarke G. Race-specific WBC and neutrophil count reference intervals. Int J Lab Hematol. 2010;32:590-7.
28. Nieto FJ, Szklo M, Folsom AR, Rock R, Mercuri M. Leukocyte count correlates in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol. 1992;136:525-37.
29. Ahmed RL, Schmitz KH, Anderson KE, Rosamond WD, Folsom AR. The metabolic syndrome and risk of incident colorectal cancer. Cancer. 2006;107:28-36.
30. Prizment AE, Anderson KE, Visvanathan K, Folsom AR. Association of inflammatory markers with colorectal cancer incidence in the atherosclerosis risk in communities study. Cancer Epidemiol Biomarkers Prev. 2011;20:297-307.
31. Mississippi Cancer Registry Reporting Manual Revised 2011. Manual2011.
32. Prizment AE, Folsom AR, Dreyfus J, Anderson KE, Visvanathan K, Joshu CE, et al. Plasma C-reactive protein, genetic risk score, and risk of common cancers in the Atherosclerosis Risk in Communities study. Cancer Causes Control. 2013;24:2077-87.
33. Howard G, Wagenknecht LE, Burke GL, Diez-Roux A, Evans GW, McGovern P, et al. Cigarette smoking and progression of atherosclerosis: The Atherosclerosis Risk in Communities (ARIC) Study. JAMA. 1998;279:119-24.

34. Kan H, Heiss G, Rose KM, Whitsel EA, Lurmann F, London SJ. Prospective analysis of traffic exposure as a risk factor for incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study. Environ Health Perspect. 2008;116:1463-8.
35. Nabulsi AA, Folsom AR, Szklo M, White A, Higgins M, Heiss G. No association of menopause and hormone replacement therapy with carotid artery intima-media thickness. Atherosclerosis Risk in Communities (ARIC) Study Investigators. Circulation. 1996;94:1857-63.
36. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol. 2010;11:889-96.
37. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell. 2006;124:263-6.
38. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140:883-99.
39. Mantovani A, Sica A, Locati M. Macrophage polarization comes of age. Immunity. 2005;23:344-6.

Table 5.1 Baseline characteristics by tertile of monocyte count in ARIC participants, 1987-1989

	Monocyte Count (10^9 cells/L)		
	Tertile 1 (0.03, 0.272)	**Tertile 2** (0.273, 0.399)	**Tertile 3** (0.400, 1.820)
Total, N	3,458	3,397	3,423
Study Site[a], %			
Jackson, MS	37.5	29.4	27.4
Forsyth County, NC	31.3	38.8	31.8
Minneapolis, MN	31.2	31.7	40.8
Male, %	36.7	44.7	54.1
Blacks, %	43.0	34.1	30.6
Age (years), Mean (SD)	53.6 (5.7)	53.9 (5.7)	54.2 (5.9)
Education, %			
<High school	23.0	21.7	21.1
High school or college graduate	39.2	38.9	39.0
Graduate school	37.8	39.5	39.9
Missing, N	4	7	6
BMI (kg/m^2), %			
<18.5	0.7	0.7	0.9
18.5-24.99	32.4	33.1	29.9
25.0 29.99	39.0	39.1	41.1
30.0-34.99	18.4	18.1	19.3
≥35.00	9.4	9.0	8.9
Missing, N	2	4	3
Waist Circumference (cm), Mean (SD)	95.8 (14.0)	96.7 (13.8)	97.4 (13.9)
Missing, N	2	0	1
Cigarette Smoking Status, %			
Never	48.9	42.2	31.5
Former	30.8	32.8	33.9
Current	20.4	25.0	34.6
Missing, N	1	5	2
Pack-Years[b], Mean (SD)	23.8 (21.8)	24.1 (21.6)	25.5 (22.1)
Missing, N	59	75	56
ETS[c] (hours/week), %			
≤1	31.6	30.3	27.1
>1	68.4	69.7	72.9
Missing, N	23	17	20
Alcohol Intake[d], Mean (SD) (g/wk)	100.4 (110.1)	111.0 (135.6)	118.1 (140.8)
Missing, N	23	21	24
CVD[e], %	8.0	9.4	11.9
Hypertension[f], %	36.0	34.6	37.3
Missing, N	1	0	2
Diabetes[g], %	11.7	10.9	12.8

Table 5.1 (continued) Baseline characteristics by tertile of monocyte count in ARIC participants, 1987-1989

	Monocyte Count (10^9 cells/L)		
	Tertile 1 (0.03, 0.272)	**Tertile 2** (0.273, 0.399)	**Tertile 3** (0.400, 1.820)
Missing, N	5	6	3
Aspirin used[h]	44.2	43.8	47.1
Missing, N	28	25	28

Abbreviations: BMI, body mass index; SD, standard deviation; ETS, environmental tobacco smoke; CVD, cardiovascular disease; HRT, hormone replacement therapy. [a]Participants from Washington County, MD were excluded from analyses due to substantial missing WBC subtype count data at this site. [b]Pack-years among ever smokers only. Pack-years was calculated as the average number of cigarettes smoked per day multiplied by the number of years of smoking and divided by 20. [c]ETS defined as the average number of hours per week of close contact with people when they are smoking. [d]Alcohol intake among participants who reported usually having at least one drink per week. [e]CVD defined as having a history of angina pectoris, coronary heart disease, intermittent claudication, or stroke. [f]Hypertension defined as use of any hypertensive medications, systolic blood pressure $\geq$140 mmHg, or diastolic blood pressure $\geq$90 mmHg. [g]Diabetes defined as having a fasting glucose level $\geq$126 mg/dl, non-fasting glucose level $\geq$200 mg/dl, a physician diagnosis of diabetes, or using sugar-lowering medications in two weeks prior to enrollment. [h]Aspirin use in two weeks prior to study enrollment.

Table 5.2 Multivariable-adjusted hazard ratios for cancer incidence by tertile of monocyte count, stratified by race, sex and cigarette smoking status, in the ARIC study, 1987-2006

Monocyte count by tertiles	N	Person-Years	Age-Standardized Rate[a]	HR (95% CI)[b]	HR (95% CI)[c, d]
Overall					
1	670	55396.4	207.1	1.00 (Reference)	1.00 (Reference)
2	696	54027.7	212.2	1.01 (0.91, 1.13)	0.98 (0.87, 1.10)
3	758	52618.0	216.6	1.01 (0.91, 1.12)	0.98 (0.86, 1.11)
P-Trend				0.88	0.74
Whites					
1	414	32371.1	218.9	1.00 (Reference)	1.00 (Reference)
2	462	36496.5	215.7	0.97 (0.85, 1.11)	0.92 (0.79, 1.08)
3	550	37153.9	229.8	0.99 (0.87, 1.13)	0.97 (0.82, 1.14)
P-Trend				0.90	0.77
Blacks					
1	256	23025.4	186.1	1.00 (Reference)	1.00 (Reference)
2	234	17531.2	206.1	1.10 (0.92, 1.31)	1.06 (0.87, 1.28)
3	208	15464.1	193.1	1.02 (0.84, 1.23)	0.95 (0.76, 1.17)
P-Trend				0.79	0.58
Males					
1	300	19798.4	245.5	1.00 (Reference)	1.00 (Reference)
2	387	23511.1	268.4	1.08 (0.93, 1.26)	1.08 (0.91, 1.28)
3	459	27409.1	245.1	0.98 (0.84, 1.14)	0.93 (0.78, 1.12)
P-Trend				0.69	0.33
Females[e]					
1	370	35598.0	175.4	1.00 (Reference)	1.00 (Reference)
2	309	30516.5	165.9	0.93 (0.80, 1.08)	0.86 (0.72, 1.02)
3	299	25208.9	193.1	1.04 (0.89, 1.22)	1.01 (0.84, 1.22)
P-Trend				0.68	0.93
Current Smokers					
1	160	10698.2	250.1	1.00 (Reference)	1.00 (Reference)
2	196	12839.0	250.0	1.02 (0.82, 1.26)	0.95 (0.76, 1.20)
3	315	17210.7	261.8	1.09 (0.90, 1.33)	0.99 (0.76, 1.20)
P-Trend				0.36	0.97
Former Smokers					
1	216	17084.7	216.1	1.00 (Reference)	1.00 (Reference)
2	235	17781.1	219.0	1.02 (0.84, 1.22)	1.05 (0.85, 1.30)
3	258	18133.5	210.2	1.03 (0.86, 1.24)	1.04 (0.83, 1.30)
P-Trend				0.73	0.75
Never Smokers					
1	294	27613.5	183.0	1.00 (Reference)	1.00 (Reference)
2	264	23366.3	187.3	1.02 (0.86, 1.21)	0.96 (0.79, 1.16)
3	185	17253.7	177.8	0.91 (0.75, 1.09)	0.90 (0.72, 1.13)
P-Trend				0.35	0.35

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Incidence rate per 1,000 individuals from 1987 to 2006 standardized to the age, race and sex distribution of the analytic cohort. [b]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-

Table 5.2 (continued) Multivariable-adjusted hazard ratios for cancer incidence by monocyte count, stratified by race, sex and cigarette smoking status, in the ARIC study, 1987-2006

29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke (≤1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [c]Additionally adjusted for neutrophil count (tertiles), lymphocyte count (tertiles), eosinophil count (tertiles) and basophil count (binary). [d]Conducted among subset with available measures of all WBC subtpes (N=10,054). [e]Additionally adjusted for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user).

Table 5.3 Multivariable-adjusted hazard ratios for cancer mortality by tertile of monocyte count, stratified by race, sex and cigarette smoking status, in the ARIC study, 1987-2008

Monocyte count by tertiles	N	Person-Years	Age-Standardized Rate[a]	HR (95% CI)[b]	HR (95% CI)[c, d]
Overall					
1	278	68689.1	83.9	1.00 (Reference)	1.00 (Reference)
2	278	66480.1	84.9	0.95 (0.80, 1.12)	0.87 (0.71, 1.05)
3	387	64638.7	109.1	1.13 (0.97, 1.33)	0.98 (0.82, 1.18)
P-Trend				0.07	0.99
Whites					
1	150	40557.1	80.0	1.00 (Reference)	1.00 (Reference)
2	170	45208.7	79.2	0.95 (0.76, 1.19)	0.88 (0.68, 1.13)
3	276	46014.0	111.5	1.21 (0.98, 1.48)	1.04 (0.82, 1.34)
P-Trend				0.03	0.56
Blacks					
1	128	28131.9	90.9	1.00 (Reference)	1.00 (Reference)
2	108	21271.4	95.0	0.93 (0.72, 1.21)	0.85 (0.64, 1.12)
3	111	18624.7	104.9	1.01 (0.77, 1.31)	0.88 (0.66, 1.19)
P-Trend				0.92	0.45
Males					
1	138	24608.3	110.4	1.00 (Reference)	1.00 (Reference)
2	155	29045.9	107.2	0.90 (0.72, 1.14)	0.87 (0.68, 1.13)
3	242	33621.1	126.6	1.00 (0.81, 1.24)	0.93 (0.72, 1.20)
P-Trend				0.80	0.70
Females[d]					
1	140	44080.7	62.1	1.00 (Reference)	1.00 (Reference)
2	123	37434.2	66.5	0.96 (0.75, 1.23)	0.82 (0.62, 1.07)
3	145	31017.6	94.8	1.29 (1.02, 1.64)	1.03 (0.78, 1.36)
P-Trend				0.03	0.79
Current Smokers					
1	102	12914.1	150.0	1.00 (Reference)	1.00 (Reference)
2	102	15638.0	132.0	0.80 (0.61, 1.06)	0.74 (0.55, 1.00)
3	207	20736.1	172.8	1.14 (0.90, 1.46)	1.04 (0.79, 1.37)
P-Trend				0.09	0.41
Former Smokers					
1	81	21242.1	78.9	1.00 (Reference)	1.00 (Reference)
2	97	21912.2	90.3	1.12 (0.83, 1.51)	1.09 (0.78, 1.51)
3	123	22342.0	100.5	1.29 (0.96, 1.72)	1.03 (0.73, 1.45)
P-Trend				0.08	0.90
Never Smokers					
1	95	34532.9	56.2	1.00 (Reference)	1.00 (Reference)
2	78	28869.7	56.6	0.99 (0.73, 1.34)	0.85 (0.60, 1.19)
3	57	21537.5	51.1	0.84 (0.60, 1.18)	0.73 (0.49, 1.09)
P-Trend				0.33	0.12

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Mortality rate per 1,000 individuals from 1987 to 2008 standardized to the age, race and sex distribution of the analytic cohort. [b]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-

Table 5.3 (continued) Multivariable-adjusted hazard ratios for cancer mortality by tertile of monocyte count, stratified by race, sex and cigarette smoking status, in the ARIC study, 1987-2008

29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke ($\leq$1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [c]Additionally adjusted for neutrophil count (tertiles), lymphocyte count (tertiles), eosinophil count (tertiles) and basophil count (binary). [d] Conducted among subset with available measures of all WBC subtpes (N=10,191). [e]Additionally adjusted for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user).

Table 5.4 Multivariable-adjusted hazard ratios for site-specific cancer incidence and mortality by monocyte count in the ARIC study, 1987-2008

Monocyte count by tertiles	Cancer Incidence			Cancer Mortality		
	N	Person-Years	HR (95% CI)[a]	N	Person-Years	HR (95% CI)[a]
Lung						
1	64	55396.4	1.00 (Reference)	69	68689.1	1.00 (Reference)
2	65	54027.7	1.03 (0.92, 1.15)	72	66480.1	0.90 (0.64, 1.25)
3	144	52618.0	0.95 (0.85, 1.07)	145	64638.7	1.38 (1.03, 1.85)
P-Trend			0.41			0.01
Colorectal						
1	73	55396.4	1.00 (Reference)	16	68689.1	1.00 (Reference)
2	66	54027.7	0.92 (0.66, 1.29)	8	66480.1	0.51 (0.22, 1.20)
3	70	52618.0	0.91 (0.65, 1.28)	17	64638.7	0.99 (0.49, 2.02)
P-Trend			0.61			0.99
Female Breast[b]						
1	145	35598.0	1.00 (Reference)	22	44080.7	1.00 (Reference)
2	119	30516.5	0.92 (0.72, 1.18)	22	37434.2	1.23 (0.68, 2.24)
3	109	25208.9	1.02 (0.79, 1.32)	17	31017.6	1.17 (0.61, 2.22)
P-Trend			0.92			0.61
Prostate						
1	128	19750.6	1.00 (Reference)	13	24559.4	1.00 (Reference)
2	173	23455.4	1.18 (0.94, 1.48)	13	28989.1	0.85 (0.39, 1.86)
3	172	27354.7	1.00 (0.79, 1.26)	15	33566.1	0.88 (0.40, 1.91)
P-Trend			0.90			0.75

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Models adjusted for study site (Jackson, Forsyth County, Minneapolis), race (white, black), body mass index (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, >35.0 kg/m2), waist circumference (continuous), education level (<high school diploma, high school diploma, >high school or college graduate, graduate school) smoking status (never, former, current), pack-years (continuous), environmental tobacco smoke ($\leq$1, >1 hour/week), alcohol intake (continuous), aspirin use in the two weeks prior to blood draw (yes/no), medical history (yes/no) of cardiovascular disease, hypertension, and diabetes. [b]Additionally adjusted for menopausal status and HRT use (pre/peri-menopausal, postmenopausal and current HRT user, postmenopausal and former HRT user, postmenopausal and never HRT user).

Chapter 6. Cytomegalovirus IgG antibodies and risk of cancer mortality in the third National Health and Nutrition Examination Survey (NHANES III)

Abstract

A role for cytomegalovirus (CMV), an endemic beta herpes virus, in tumor development and progression is postulated based on laboratory studies. In this study, we examined the prospective association between CMV IgG level, a proposed marker of subclinical CMV reactivation, and subsequent cancer mortality among CMV seropositive adults, ages 40 to 70 years, in the third National Health and Nutrition Examination Survey (NHANES III) (N=5,063). Levels of CMV IgG were measured at baseline, between 1988 and 1994, and information on cause-specific mortality was available through December 31, 2011 through linkage with the National Death Index. Multivariable-adjusted hazard ratios (HR) and 95% confidence intervals (CI) for cancer mortality were estimated by binary category of CMV IgG level. During follow-up, 505 cancer deaths occurred. In the overall cohort, there was no association between CMV IgG level and cancer mortality (HR: 0.89, 95% CI: 0.67, 1.18). However, in analyses stratified by race, black participants with high CMV IgG had a 38% (HR: 1.38, 95% CI: 1.02, 1.89) increased risk of cancer mortality, while there was no association in whites (HR: 0.74, 95% CI: 0.53, 1.04) or Mexican Americans (HR: 1.00, 95% CI: 0.60, 1.67) (p-interaction term=0.02). Among blacks, a significant association between CMV IgG level and cancer mortality persisted after excluding cancer cases within five years of baseline blood draw. Our findings support a link between the host immune response mounted against CMV and cancer mortality in immunocompetent black adults. Future studies are warranted to explore the mechanism underlying this association and the differences by race further.

Introduction

Cytomegalovirus (CMV) is an endemic beta herpes virus with seroprevalence estimates in the U.S. ranging from 36.6% in 6 to 11 years olds to 90.8% in adults 80 years and older (1). Immunocompetent individuals infected with CMV mount an extensive and life-long immune response to ensure viral latency (2). Post infection, a low-level CMV IgG antibody titer is established which persists throughout the lifetime and may vary depending on the frequency and intensity of subclinical viral reactivation (3-5). While CMV infection is not associated with clinically overt symptoms in immunocompetent individuals, there is mounting evidence from prospective epidemiological studies supporting an association between CMV infection and/or IgG level and several chronic health conditions, including cardiovascular disease (CVD) (6-10), cancer (11, 12), frailty (13, 14), and cognitive decline (15), and all cause (6, 13, 16-19) and CVD mortality (9, 16, 17).

Cytomegalovirus (CMV) viral products may directly promote tumor development and progression by altering the expression of factors regulating cell survival, replication, motility and adhesion (20-25). Another mechanism by which CMV may exert carcinogenic effects is by altering host immunity. In in vitro studies CMV has been shown to induce immune suppression (26, 27), which may compromise tumor immunosurveillance, the process by which the host immune system detects and eliminates premalignant and malignant cells (28). Subclinical CMV reactivation may also deregulate the host inflammatory response resulting in systemic and localized inflammation (23), an etiological factor for several types of cancer (29-31).

Previous prospective studies have evaluated the relationship between CMV serostatus (seropositive versus seronegative) and breast and prostate cancer incidence; in these studies, no associations were found (11, 32, 33). Other studies have quantified the risk of cancer incidence and mortalty by CMV IgG levels. In a small study of young women, high CMV IgG was associated with an increased risk of invasive breast cancer, compared to lower CMV IgG levels (11). Interestingly, in this study, CMV seropositivity, compared to seronegativity, was not associated with breast cancer risk (11). In a subsequent study, a significant increase in CMV IgG level in the years preceding breast cancer was found in this same cohort (12). Only one study has reported the association between CMV IgG levels and cancer mortality. In this large prospective cohort, high CMV IgG levels were associated with a non-significant increased risk of cancer mortality compared to seronegative persons. Among CMV seropositive persons, however, there was no difference in the risk of cancer mortality by level of CMV IgG (6).

In this study, we prospectively evaluated the association between CMV IgG levels and cancer mortality among CMV seropositive persons in a U.S. population-based study, the third National Health and Nutrition Examination Survey (NHANES III). We hypothesized that more active CMV infection, as indicated by higher levels of CMV IgG, would be associated with an increased risk of cancer mortality.

Materials and Methods

Study population

The third National Health and Nutrition Examination Survey (NHANES III) is a nationally representative study population of non-institutionalized, civilian, United States citizens aged two months and older, recruited between 1988 and 1994. Study participants were identified through a complex, multi-stage, clustered, stratified sampling approach, with oversampling of African Americans, Mexican Americans, children (< 5 years) and the elderly (> 60 years). Sample weights based on the U.S. Census Bureau population estimates at the time were generated for each person to provide nationally representative estimates of health conditions. A priori, the analytic cohort was limited to adults, ages 40 to 70 years in order to minimize the confounding effects of age, given that age is a major determinant of CMV IgG level, particularly among older age groups (1, 34). Participants included in the analytic cohort attended the mobile examination center (MEC) and met the following eligibility criteria: (1) no missing data for CMV serostatus, CMV IgG OD level, or cause-specific mortality (N=6,362); (2) no personal history of cancer except skin carcinoma (N=6,102); (3) not pregnant at the time of blood draw, given that pregnancy alters immune response (N=6,087); and (4) cause of death not due to HIV in order to restrict the study population to immunocompetent healthy adults (N=6,078). The main analyses were additionally limited to CMV seropositive individuals as there were a limited number of CMV seronegative persons in the age range of 40 to 70 years (N=5,063).

Exposure measurement

Cytomegalovirus IgG optical density (OD) (arbitrary units (AU)/ml) was measured in stored, frozen (-20°C) sera samples collected at baseline using an Enzyme Linked

Immunoassay (ELISA) (Quest International, Inc., Miami, FL) in 2006. Cytomegalovirus IgG OD values greater than 1.05 AU/ml were determined to be seropositive. Because this assay was conducted by NHANES III to determine serostatus and not to quantify CMV IgG levels, approximately one-third of CMV IgG levels were arbitrarily right-censored per batch. Among CMV seropositive persons in this analytic cohort, a total of 50% of IgG values were right-censored. Access to the continuous IgG OD values requires special permission from the National Center for Health Statistics (NCHS).

Mortality follow-up

Vital status and date of cause-specific mortality were available as of December 31, 2011 through linkage with the National Death Index (NDI). Access to this data requires special permission from the NCHS. Cause of death was determined using the underlying cause of death on death certificates and classified according to the *International Classification of Diseases, 10th Revision* (ICD-10). Deaths occurring before 1999 were recoded according to the ICD-10 system by NCHS (35). The ICD-10 codes, C00-C97, indicated cases of cancer-specific mortality. In analyses, follow-up time accrued from the date of baseline blood draw, between 1988 and 1994, until the first of the following events occurred: (1) date of cancer death or (2) administrative censoring on December 31, 2011.

Covariates assessment

At baseline, study participants completed a series of detailed questionnaires, underwent a clinical examination and provided a blood sample. Self-reported information on age, race, education attainment, marital status, annual family income, and health insurance

coverage (yes/no), including Medicare, Medicaid and private insurance, was based on self-report. Medicare was collected. Participants were categorized as residing near a population of one million people or not, based on the U.S. Department of Agriculture urban/rural code. The household crowdedness index was calculated as the number of persons dwelling in a residence divided by the number of rooms and categorized as $\leq$0.5, >0.5 to 1, >1 (36). Having regular health care access was defined as having at least one usual source of medical care in case of sickness or for routine care. Cigarette smoking status was categorized as never (<100 cigarettes), former ($\geq$100 cigarettes and not currently smoking), and current. Among ever smokers, the average number of cigarettes smoked (currently or at the time of quitting) and the total years of smoking were used to calculate pack-years ([cigarettes per day*years smoking]/20 cigarettes per pack). Trained technicians at mobile examination centers obtained anthropometric measurements, including weight, height, and waist circumference. Weight and height measurements were used to calculate body mass index (BMI) (weight (kg)/[height (m)]^2). A medical history of CVD was defined as having had a heart attack, stroke, or congestive heart failure. Hypertension was defined as either a physician diagnosis, systolic blood pressure $\geq$140 mm/Hg, diastolic blood pressure $\geq$90 mm/Hg, or use of anti-hypertensive medications. Participants were classified as being diabetic if they reported a physician diagnosis or used any sugar-lowering medications in 30 days prior to enrollment. Women were categorized as being postmenopausal if they did not have their menstrual period in the past 12 months. Women missing information for this variable was categorized as being postmenopausal if they were 51 years of age or older. Postmenopausal hormone use was defined as use of oral estrogen or female hormone pill other than oral

contraceptives. Non-prescription and prescription non-steroidal anti-inflammatory drug (NSAID) use in the 30 days prior to blood draw were based on self-report and medical container review by NHANES III staff. Baseline levels of C-reactive protein (CRP) (<0.3, ≥0.3 mg/dl) were measured using latex-enhanced nephelometry.

Statistical Analyses

All statistical analyses incorporated the appropriate sample weights to account for the sampling approach. Cytomegalovirus (CMV) seropositive participants were categorized as having low CMV IgG levels, defined as being within the arbitrary limit of detection, or high CMV IgG levels. Means and proportions of baseline covariates were compared across categories of CMV IgG level using linear regression for continuous values and the chi-squared test for categorical values.

Cox proportional hazards models were used to estimate the hazard ratio (HR) and 95% confidence interval (CI) of cancer specific mortality by category of CMV IgG level. Multivariable-adjusted models included the baseline covariates, age, sex, race (non-Hispanic white, non-Hispanic black, Mexican American, other), education attainment (<high school diploma, high school diploma, > high school), marital status (married/cohabitating, widowed/separated/divorced, never married), census region (Northeast, Midwest, South, West), country of origin (U.S., foreign), annual family income (<$20,000, ≥$20,000), cigarette smoking status (never, former, current), pack-years (continuous), BMI (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m^2), health insurance coverage (yes/no), medical history (yes/no) of CVD and diabetes, NSAID use

in past month (yes/no), and CRP level (<0.3, ≥0.3 mg/dl). Missing values for pack-years were replaced with the median value and missing categorical data were replaced with an indicator variable. Urban/rural index, house crowdedness index, usual health care, waist circumference, hypertension, and menopausal status were not associated with CMV IgG level and, therefore, were not included in final multivariable-adjusted models. Effect modification between CMV IgG category and select factors which may modulate CMV pathogenesis, including sex, race, cigarette smoking status, BMI, and NSAID use, were assessed by introducing a cross-product term in models and using the Wald test to assess statistical significance. The proportional hazards assumption was assessed by introducing a cross-product term between CMV IgG level and follow-up time into models. In all cases, the interaction term was not statistically significant.

Exploratory, cancer mortality site-specific analyses were conducted for the four most common cancers (i.e., female breast, colorectal, lung, and prostate cancers). Additionally, the following sensitivity analyses were performed: (1) excluding cancer deaths within one and five years of baseline blood draw to address any bias due to reverse causality; (2) excluding cancer deaths due to leukemia or lymphoma, which directly affect immune cells, as there may be a differential association between CMV and blood cancers compared to the more common epithelial cancers; (3) excluding individuals with a medical history of autoimmune or inflammatory diseases, including lupus and rheumatoid arthritis, and recent users of NSAIDs, as these conditions/drugs may alter CMV pathogenesis; and (4) excluding individuals with a history of skin carcinoma at baseline as the type of skin cancer (basal cell versus melanoma) was not specified. We

also compared CMV IgG levels to the reference group, CMV seronegative persons; however, these analyses are considered exploratory due to the small number of CMV seronegative persons. All analyses were conducted using STATA version 11.2 (Stata Corporation, College Station, TX, 2012).

Results

Of the total 5,063 seropositive men and women, 50% had detectable levels of CMV IgG antibody (mean: 2.13 OD units, 95% CI: 2.08, 2.17), and 50% had antibody levels above the arbitrary threshold designated per batch, and were therefore right-censored. Table 6.1 presents the distribution of baseline characteristics by CMV IgG antibody level. Compared to persons with low CMV IgG antibody levels, seropositive persons with high titers, were slightly older; more likely to be female; more likely to be black; more likely to be less educated; more likely to have an annual family income <$20,000; more likely to be uninsured; more likely to be a current cigarette smoker; more likely to report a diagnosis of CVD and diabetes; and more likely to have CRP levels $\geq$0.3. There were no differences in BMI, waist circumference, pack-years, menopausal status, or NSAID use by level of CMV IgG. Additionally, geographic characteristics, such as census region and urban residence, did not vary by CMV IgG category. The distribution of baseline characteristics by level of CMV IgG in whites, blacks and Mexican Americans are shown in Appendix D, Tables D.1-D.3. In Mexican Americans, current smokers were more likely to have high levels of CMV IgG while there was a suggestive trend in whites and no difference in blacks. Unlike in whites, blacks and Mexican Americans with high CMV IgG were more likely to also have high levels of CRP and, among Mexican Americans, a

greater proportion of uninsured persons had high levels of CMV IgG, while there was no difference in whites or blacks.

Between 1988 and 2011, 505 cancer deaths occurred during a total of 86,213 person-years. The leading causes of cancer deaths were lung (N= 175), colorectal (N=36), female breast (N=37) and prostate (N=34) cancer. Table 6.2 presents the multivariable-adjusted HRs of cancer mortality by level of CMV IgG among CMV seropositive individuals. Overall, higher CMV IgG was not associated with an increased risk of cancer mortality, compared to low CMV IgG levels (HR: 0.89, 95% CI: 0.67, 1.18). However, among blacks, high CMV IgG was associated with a 38% (HR: 1.38, 95% CI: 1.02, 1.89) increased risk of cancer mortality, while there was a marginal inverse association in whites (HR: 0.74, 95% CI: 0.53, 1.04) and no association in Mexican Americans (HR: 1.00, 95% CI: 0.60, 1.67) (all p-interaction terms$\leq$ 0.02). There was no evidence of a statistical interaction between category of CMV IgG and select factors, which may modulate CMV pathogenesis, including sex, cigarette smoking status, BMI, or recent NSAID use (all p-interaction terms $\geq$0.3).

In sensitivity analyses, a significant positive association among blacks remained after excluding cancer deaths within five years of baseline blood draw, persons who reported using NSAIDs in the month prior to baseline, and persons with a medical history of lupus and skin cancer at baseline (Appendix D, Table D.4). A positive association was present, but no longer statistically significant, after removing persons with a history of rheumatoid arthritis (Appendix D, Table D.4). We also examined the leading causes of cancer death

by race to determine if the differential association by race may be due to differences in the types of cancer affecting these groups; No significant differences were found in the types of cancer deaths in whites, blacks or Mexican Americans (p-value=0.58; Appendix D, Figure D.1). Additionally, there was no association between high CMV IgG and non-cancer mortality in blacks.

In cancer mortality site-specific analyses, high CMV IgG was not associated with female breast, colorectal, lung or prostate cancer (Table D.3). We had limited power to conduct cancer mortality site-specific analyses by race. Nevertheless, among blacks, there was a suggestive positive association between high CMV IgG and lung cancer mortality (HR: 1.54, 95% CI: 0.96, 2.46) compared to low CMV IgG. Excluding cancer deaths due to leukemia or lymphoma did not alter risk estimates.

Figure D.1 shows the risk of cancer mortality in CMV seropositive persons, overall, and by level of CMV IgG, relative to CMV seronegative persons. In multivariable-adjusted models, there was no association between CMV serostatus and cancer mortality (HR: 1.25, 95% CI: 0.83, 1.88) nor was there any association in persons with low (HR: 1.32, 95% CI: 0.84, 2.09) or high CMV IgG (HR: 1.16, 95% CI: 0.78, 1.74). Given the low number of cancer deaths among CMV seronegatives, and particularly in blacks (N=11) and Mexican Americans (N=4), further stratification by race may result in unstable risk estimates. Nevertheless, in exploratory analyses, blacks with higher CMV IgG levels had an 89% (HR: 1.89, 95% CI: 1.09, 3.30) increased risk of cancer mortality compared to CMV seronegatives, while there was no association between low CMV IgG and cancer

mortality (HR: 1.35, 95% CI: 0.71, 2.57, p-trend=0.003). Additionally, no associations were found for either high or low CMV IgG in whites and Mexican Americans (Appendix D, Table D.5).

Discussion

In this U.S. population-based prospective study, there was no association between CMV IgG level and cancer mortality overall, however black participants with high CMV IgG levels had a significantly increased risk of total cancer mortality, compared to black CMV seropositive participants with low CMV IgG. In exploratory analyses, blacks with high CMV IgG antibody titer also had an increased risk of cancer mortality compared to black CMV seronegative persons. No associations were found in whites or Mexican Americans. The significant positive association between CMV IgG level and cancer mortality among black seropositive participants persisted after excluding cancer deaths within five years of baseline blood draw to account for the presence of subclinical disease. Additionally, we did not find any significant difference in the leading causes of cancer mortality in whites, blacks and Mexican Americans. Thus, the differential association between CMV IgG level and cancer mortality by race does not seem to be driven by a specific cancer type. Overall, our findings support an association between the host immune response mounted against CMV and cancer mortality in immunocompetent black adults.

Only one prospective study, conducted in the EPIC-Norfolk study, has previously evaluated the association between CMV IgG level and cancer mortality. Consistent with

our findings, in this cohort study of over 13,000 men and women, the highest tertile of CMV IgG was not associated with cancer mortality among CMV seropositive persons (6). However in this previous study, set in the United Kingdom, differential associations by race could not be evaluated. Additionally, a limitation of this previous study was the inclusion of persons with a history of cancer at baseline. Thus, the levels of CMV IgG may reflect the effects of cancer and/or treatment rather than an individual's baseline CMV activity, positively biasing risk estimates. However, in this case, this does not seem to be a major source of bias given that a null association was reported between CMV IgG level and cancer mortality.

The mechanism underlying our finding of a differential relationship between CMV IgG and cancer mortality in blacks compared to whites and Mexican Americans is not known. Nevertheless, our finding of a positive association between CMV IgG level and cancer mortality among blacks may provide indirect support for a causal role for CMV in carcinogenesis. This is plausible based on several lines of evidence implicating CMV in oncomodulation, via the infection tumor cells (22, 23). In clinical studies, CMV has been detected in tumor cells, but not in the surrounding tissue, in cases of prostate (37), colon (38), cervical (39), and breast (40) cancers. Additionally, findings from in vitro and in vivo studies suggest a direct role for CMV in carcinogenesis. In these studies, CMV viral products have been shown to affect cell survival, proliferation and differentiation, and gene expression (41-44). Cytomegalovirus (CMV) may also promote cancer development and progression indirectly by deregulating the inflammatory and cell-mediated immune responses (23).

It is also possible that increased CMV activity is a surrogate marker of suppressed immunity and inflammation, rather than a causal factor. Specifically, CMV reactivation has been shown to be dependent on host immunity (45, 46). Indeed, even subclinical changes in immune function may affect CMV reactivation. This is supported by several studies, which have reported higher CMV IgG levels in the elderly (4), astronauts in space (47), and persons experiencing stress (48). Thus, CMV IgG levels may be a marker of general immune dysfunction, unrelated to the downstream effects of CMV pathogenesis.

Additionally, we cannot discount the potential effects of unmeasured or residual confounding by other factors in our analyses. In particular, given the strong association between CMV IgG level and socioeconomic status (3), it is plausible that participants with high CMV IgG levels also have poorer health behaviors and access to care, resulting in less frequent cancer screening, higher stage and grade at diagnosis, and less aggressive treatment options. Importantly, however, although confounding by these unmeasured factors may result in a spurious positive association between CMV IgG and cancer mortality, it is unlikely that such confounding would be differential by race. Furthermore, if the association between CMV IgG and cancer mortality were explained by confounding due to socioeconomic factors, one would expect a similar positive association with non-cancer mortality, however this was not observed.

Previous studies examining the relationship between CMV and all-cause, CVD, and cancer mortality have not evaluated differences by race (6, 9, 13, 16-19). One possible explanation for the differential association reported in this study may be that genetic variant(s) in persons of African American descent directly or indirectly affect CMV pathogenesis. Indeed, several studies have identified genetic polymorphisms associated with differences in host immunity in blacks compared to whites (49-53). Importantly though, this explanation does not clarify the differential effect of CMV by race in the context of cancer mortality specifically. Alternatively, race may be a marker of unmeasured social/cultural factors, which modify the association between CMV IgG and cancer mortality. To explore this explanation further, future studies, with adequate sample size to stratify by both race and factors such as smoking, BMI and NSAIDs use, may be informative.

In the present study, the main analyses were conducted among CMV seropositive participants, with the reference group being CMV seropositive individuals with low CMV IgG levels. As such, our analyses evaluate the association between the host immune response to CMV IgG and cancer mortality. Due to the small number of CMV seronegative participants in the predefined age range (40 to 70 years), we had limited power to compare CMV IgG levels to persons who were CMV seronegative, particularly after racial stratification. This limitation is not specific to this study, but rather is an issue for all U.S. based cohort studies, given that the majority of adults over the age of 40 years are CMV seropositive and CMV seroprevalence reaches nearly 100% in the very elderly (1). In addition to issues of power, CMV seronegative persons may also have major

differences with respect to age, sociodemographic factors, health behaviors, and medical history, compared to CMV seropositive persons. Thus, inclusion of this comparator group may introduce bias due to residual confounding.

Our findings should be interpreted in light of several limitations. First, we utilized a one-time measure of CMV IgG, precluding the assessment of time-varying changes. In NHANES III, among a subset of women, ages 12 to 49 years, the prevalence of recent CMV infection, re-infection or re-activation, based on levels of IgM and IgG avidity, was 3% or lower (54). Thus, it is unlikely that we have included IgG levels transiently elevated due to recent, acute infection. Nevertheless, the stability of CMV IgG as a marker of *established* infection has not been well evaluated. Second, although CMV IgG is a widely proposed marker of CMV activity (3-5, 17), there is no direct evidence to support this. Third, in the total NHANES III study population, approximately one third of CMV IgG levels were right-censored per batch. Although, there was not a single maximum detectable threshold, we would not expect significant differences in the distribution of CMV IgG per batch, and, thus, the maximum detectable limits are unlikely to vary significantly by batch as well. Furthermore, any misclassification that may have resulted from this censoring approach would be non-differential, biasing our findings towards the null. Another consequence of the right censoring of IgG levels is that we were unable to explore finer categorizations of CMV IgG level. Fourth, we had limited power to detect associations with site-specific cancers and to explore effect modification among blacks. Finally, because information on cancer incidence was not available, we were unable to explore the role of CMV in tumor initiation and promotion or the impact

of CMV infection on stage and grade of disease. Strengths of this study include the use of a large, U.S. population-based cohort, with a significant proportion of black participants, detailed information on demographic and lifestyle risk factors, including socioeconomic status, and highly complete data on cause-specific mortality collected over 23 years of follow-up.

In conclusion, we report an association between high CMV IgG level and an increased risk of cancer mortality in CMV seropositive blacks, but not whites or Mexican Americans. These findings suggest that more active CMV infection, as measured by CMV IgG antibody titer, may be associated with cancer progression. Further, the differential association between CMV and cancer mortality by race may have important implications for the disparate burden of cancer deaths in blacks, particularly given the high prevalence of CMV infection in U.S. adults. Additional studies are needed to validate our results and to explore the mechanisms underlying our findings.

References

1. Staras SA, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ. Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. Clin Infect Dis. 2006;43:1143-51.
2. Britt W. Manifestations of human cytomegalovirus infection: proposed mechanisms of acute and chronic disease. Curr Top Microbiol Immunol. 2008;325:417-70.
3. Dowd JB, Aiello AE. Socioeconomic differentials in immune response. Epidemiology. 2009;20:902-8.
4. Stowe RP, Kozlova EV, Yetman DL, Walling DM, Goodwin JS, Glaser R. Chronic herpesvirus reactivation occurs in aging. Exp Gerontol. 2007;42:563-70.
5. Stowe RP, Peek MK, Perez NA, Yetman DL, Cutchin MP, Goodwin JS. Herpesvirus reactivation and socioeconomic position: a community-based study. J Epidemiol Community Health. 2010;64:666-71.
6. Gkrania-Klotsas E, Langenberg C, Sharp SJ, Luben R, Khaw KT, Wareham NJ. Seropositivity and higher immunoglobulin g antibody levels against cytomegalovirus are associated with mortality in the population-based European prospective investigation of cancer-norfolk cohort. Clin Infect Dis. 2013;56:1421-7.
7. Simanek AM, Dowd JB, Aiello AE. Persistent pathogens linking socioeconomic position and cardiovascular disease in the US. Int J Epidemiol. 2009;38:775-87.
8. Danesh J, Collins R, Peto R. Chronic infections and coronary heart disease: is there a link? Lancet. 1997;350:430-6.
9. Smieja M, Gnarpe J, Lonn E, Gnarpe H, Olsson G, Yi Q, et al. Multiple infections and subsequent cardiovascular events in the Heart Outcomes Prevention Evaluation (HOPE) Study. Circulation. 2003;107:251-7.
10. Sorlie PD, Nieto FJ, Adam E, Folsom AR, Shahar E, Massing M. A prospective study of cytomegalovirus, herpes simplex virus 1, and coronary heart disease: the atherosclerosis risk in communities (ARIC) study. Arch Intern Med. 2000;160:2027-32.
11. Richardson AK, Cox B, McCredie MR, Dite GS, Chang JH, Gertig DM, et al. Cytomegalovirus, Epstein-Barr virus and risk of breast cancer before age 40 years: a case-control study. Br J Cancer. 2004;90:2149-52.
12. Cox B, Richardson A, Graham P, Gislefoss RE, Jellum E, Rollag H. Breast cancer, cytomegalovirus and Epstein-Barr virus: a nested case-control study. Br J Cancer. 2010;102:1665-9.
13. Wang GC, Kao WH, Murakami P, Xue QL, Chiou RB, Detrick B, et al. Cytomegalovirus infection and the risk of mortality and frailty in older women: a prospective observational cohort study. Am J Epidemiol. 2010;171:1144-52.
14. Aiello AE, Haan MN, Pierce CM, Simanek AM, Liang J. Persistent infection, inflammation, and functional impairment in older Latinos. J Gerontol A Biol Sci Med Sci. 2008;63:610-8.
15. Aiello AE, Haan M, Blythe L, Moore K, Gonzalez JM, Jagust W. The influence of latent viral infection on rate of cognitive decline over 4 years. J Am Geriatr Soc. 2006;54:1046-54.

16. Simanek AM, Dowd JB, Pawelec G, Melzer D, Dutta A, Aiello AE. Seropositivity to cytomegalovirus, inflammation, all-cause and cardiovascular disease-related mortality in the United States. PLoS One. 2011;6:e16103.
17. Roberts ET, Haan MN, Dowd JB, Aiello AE. Cytomegalovirus antibody levels, inflammation, and mortality among elderly Latinos over 9 years of follow-up. Am J Epidemiol. 2010;172:363-71.
18. Strandberg TE, Pitkala KH, Tilvis RS. Cytomegalovirus antibody level and mortality among community-dwelling older adults with stable cardiovascular disease. JAMA. 2009;301:380-2.
19. Muhlestein JB, Horne BD, Carlquist JF, Madsen TE, Bair TL, Pearson RR, et al. Cytomegalovirus seropositivity and C-reactive protein have independent and combined predictive value for mortality in patients with angiographically demonstrated coronary artery disease. Circulation. 2000;102:1917-23.
20. Soroceanu L, Cobbs CS. Is HCMV a tumor promoter? Virus Res. 2011;157:193-203.
21. Cinatl J, Scholz M, Kotchetkov R, Vogel JU, Doerr HW. Molecular mechanisms of the modulatory effects of HCMV infection in tumor cell biology. Trends Mol Med. 2004;10:19-23.
22. Michaelis M, Baumgarten P, Mittelbronn M, Driever PH, Doerr HW, Cinatl J, Jr. Oncomodulation by human cytomegalovirus: novel clinical findings open new roads. Med Microbiol Immunol. 2011;200:1-5.
23. Soderberg-Naucler C. Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer? J Intern Med. 2006;259:219-46.
24. Olsson J, Wikby A, Johansson B, Lofgren S, Nilsson BO, Ferguson FG. Age-related change in peripheral blood T-lymphocyte subpopulations and cytomegalovirus infection in the very old: the Swedish longitudinal OCTO immune study. Mech Ageing Dev. 2000;121:187-201.
25. Rinaldo CR, Jr., Carney WP, Richter BS, Black PH, Hirsch MS. Mechanisms of immunosuppression in cytomegaloviral mononucleosis. J Infect Dis. 1980;141:488-95.
26. Koch S, Solana R, Dela Rosa O, Pawelec G. Human cytomegalovirus infection and T cell immunosenescence: a mini review. Mech Ageing Dev. 2006;127:538-43.
27. Pawelec G, Akbar A, Beverley P, Caruso C, Derhovanessian E, Fulop T, et al. Immunosenescence and Cytomegalovirus: where do we stand after a decade? Immun Ageing. 2010;7:13.
28. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Adv Immunol. 2006;90:1-50.
29. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell. 2005;7:211-7.
30. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420:860-7.
31. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140:883-99.
32. Sutcliffe S, Till C, Gaydos CA, Jenkins FJ, Goodman PJ, Hoque AM, et al. Prospective study of cytomegalovirus serostatus and prostate cancer risk in the Prostate Cancer Prevention Trial. Cancer Causes Control. 2012;23:1511-8.

33. Huang WY, Hayes R, Pfeiffer R, Viscidi RP, Lee FK, Wang YF, et al. Sexually transmissible infections and prostate cancer risk. Cancer Epidemiol Biomarkers Prev. 2008;17:2374-81.
34. Dowd JB, Aiello AE, Alley DE. Socioeconomic disparities in the seroprevalence of cytomegalovirus infection in the US population: NHANES III. Epidemiol Infect. 2009;137:58-65.
35. Anderson RN, Minino AM, Hoyert DL, Rosenberg HM. Comparability of cause of death between ICD-9 and ICD-10: preliminary estimates. Natl Vital Stat Rep. 2001;49:1-32.
36. Everhart JE, Kruszon-Moran D, Perez-Perez GI, Tralka TS, McQuillan G. Seroprevalence and ethnic differences in Helicobacter pylori infection among adults in the United States. J Infect Dis. 2000;181:1359-63.
37. Samanta M, Harkins L, Klemm K, Britt WJ, Cobbs CS. High prevalence of human cytomegalovirus in prostatic intraepithelial neoplasia and prostatic carcinoma. J Urol. 2003;170:998-1002.
38. Harkins L, Volk AL, Samanta M, Mikolaenko I, Britt WJ, Bland KI, et al. Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. Lancet. 2002;360:1557-63.
39. Pacsa AS, Kummerlander L, Pejtsik B, Pali K. Herpesvirus antibodies and antigens in patients with cervical anaplasia and in controls. J Natl Cancer Inst. 1975;55:775-81.
40. Taher C, de Boniface J, Mohammad AA, Religa P, Hartman J, Yaiw KC, et al. High prevalence of human cytomegalovirus proteins and nucleic acids in primary breast cancer and metastatic sentinel lymph nodes. PLoS One. 2013;8:e56795.
41. Slinger E, Maussang D, Schreiber A, Siderius M, Rahbar A, Fraile-Ramos A, et al. HCMV-encoded chemokine receptor US28 mediates proliferative signaling through the IL-6-STAT3 axis. Sci Signal. 2010;3:ra58.
42. Kalejta RF, Bechtel JT, Shenk T. Human cytomegalovirus pp71 stimulates cell cycle progression by inducing the proteasome-dependent degradation of the retinoblastoma family of tumor suppressors. Mol Cell Biol. 2003;23:1885-95.
43. Winkler LL, Hwang J, Kalejta RF. Ubiquitin-independent proteasomal degradation of tumor suppressors by human cytomegalovirus pp71 requires the 19S regulatory particle. J Virol. 2013;87:4665-71.
44. Kalejta RF, Shenk T. Proteasome-dependent, ubiquitin-independent degradation of the Rb family of tumor suppressors by the human cytomegalovirus pp71 protein. Proc Natl Acad Sci U S A. 2003;100:3263-8.
45. Soderberg-Naucler C, Fish KN, Nelson JA. Interferon-gamma and tumor necrosis factor-alpha specifically induce formation of cytomegalovirus-permissive monocyte-derived macrophages that are refractory to the antiviral activity of these cytokines. J Clin Invest. 1997;100:3154-63.
46. Soderberg-Naucler C, Streblow DN, Fish KN, Allan-Yorke J, Smith PP, Nelson JA. Reactivation of latent human cytomegalovirus in CD14(+) monocytes is differentiation dependent. J Virol. 2001;75:7543-54.
47. Mehta SK, Stowe RP, Feiveson AH, Tyring SK, Pierson DL. Reactivation and shedding of cytomegalovirus in astronauts during spaceflight. J Infect Dis. 2000;182:1761-4.

48. Glaser R, Kiecolt-Glaser JK, Speicher CE, Holliday JE. Stress, loneliness, and changes in herpesvirus latency. J Behav Med. 1985;8:249-60.
49. Van Dyke AL, Cote ML, Wenzlaff AS, Land S, Schwartz AG. Cytokine SNPs: Comparison of allele frequencies by race and implications for future studies. Cytokine. 2009;46:236-44.
50. Cox ED, Hoffmann SC, DiMercurio BS, Wesley RA, Harlan DM, Kirk AD, et al. Cytokine polymorphic analyses indicate ethnic differences in the allelic distribution of interleukin-2 and interleukin-6. Transplantation. 2001;72:720-6.
51. Hoffmann SC, Stanley EM, Cox ED, DiMercurio BS, Koziol DE, Harlan DM, et al. Ethnicity greatly influences cytokine gene polymorphism distribution. Am J Transplant. 2002;2:560-7.
52. Reich D, Nalls MA, Kao WH, Akylbekova EL, Tandon A, Patterson N, et al. Reduced neutrophil count in people of African descent is due to a regulatory variant in the Duffy antigen receptor for chemokines gene. PLoS Genet. 2009;5:e1000360.
53. Ness RB, Haggerty CL, Harger G, Ferrell R. Differential distribution of allelic variants in cytokine genes among African Americans and White Americans. Am J Epidemiol. 2004;160:1033-8.
54. Dollard SC, Staras SA, Amin MM, Schmid DS, Cannon MJ. National prevalence estimates for cytomegalovirus IgM and IgG avidity and association between high IgM antibody titer and low IgG avidity. Clin Vaccine Immunol. 2011;18:1895-9.

Table 6.1 Baseline characteristics by levels of CMV IgG in CMV seropositive adults, ages 40 to 70 years, in the NHANES III study, 1988-1994

	Low CMV IgG	High CMV IgG
Total, N	2,526	2,537
Mean Age (years) (SE)	53.3 (0.36)	54.1 (0.38)
Male, %	53.3	37.0
Race, %		
Non-Hispanic white	74.4	71.2
Non-Hispanic black	10.9	14.4
Mexican American	10.2	9.2
Other	4.5	5.2
Census Region, %		
Northeast	19.3	18.3
Midwest	22.2	23.4
South	38.4	38.8
West	20.4	19.6
Urban Residence[a], %	48.8	45.8
Country of Origin, %		
United States	83.3	83.0
Other	16.3	16.7
Missing	0.4	0.3
Household Crowding Index[b], %		
<0.5	54.0	53.6
0.5-0.99	36.0	37.4
≥1	10.0	8.9
Missing	0.1	0.0
Education, %		
Less than high school	33.7	36.2
High school diploma	29.1	32.5
More than high school	36.5	30.8
Missing	0.6	0.6
Marital Status, %		
Married, cohabitating	74.1	70.5
Widowed, divorced, separated	20.4	24.2
Never married	5.2	5.2
Missing	0.3	0
Annual Family Income ≥$20,000, %	70.6	62.0
Missing	1.4	2.2
Uninsured[c], %	10.8	13.8
Missing	1.5	15
≥1 Usual Source of Medical Care[d], %	83.6	84.7
Body Mass Index (kg/m^2)[e], %		
<18.5	1.1	2.1
18.5-24.9	34.0	32.1
25.0-29.9	37.5	35.7
30.0-34.9	18.5	18.4
≥35.0	8.9	11.7

Table 6.1 (continued) Baseline characteristics by levels of CMV IgG in CMV seropositive adults, ages 40 to 70 years in the NHANES III study, 1988-1994

	Low CMV IgG	High CMV IgG
Missing	0.2	0.0
Waist Circumferences (cm), Mean (SE)	96.5 (0.58)	95.8 (0.38)
Missing, %	3.2	3.2
Postmenopausal[f], %	65.9	64.3
Cigarette Smoking Status, %		
Never	40.4	40.6
Former	35.0	29.5
Current	24.6	29.9
Pack-Years[g], Mean (SE)	32.9 (1.2)	34.4 (1.1)
Missing, %	2.3	3.0
Cardiovascular Disease[h], %	6.8	9.0
Hypertension[i], %	45.8	44.7
Diabetes[j], %	6.9	8.8
Missing	0	0.2
C-Reactive Protein ≥0.3 mg/dL, %	34.1	38.3
Missing	0.4	1.1
NSAID Use in Past Month[k], %	57.5	56.5

Abbreviations: SE, standard error. [a]Urban residence defined as residing near a population of one million people based on the U.S. Department of Agriculture urban/rural code. [b]Household crowding index was calculated as the number of persons dwelling in a residence divided by the number of rooms. [c]Persons were uninsured if they did not have Medicare, Medicaid or private insurance. [d]Usual source of medical care was defined as having at least one medical facility or physician to contact in case of sickness or for routine care. [e]Body mass index calculated as weight (kg) divided by height (m) squared. [f]Postmenopausal was defined as not having a menstrual period in the past 12 months. [g]Pack-year calculated among ever smokers as the number of cigarettes per day multiplied by years smoking and then divided by 20. [h]Cardiovascular disease was defined as having had a heart attack, stroke, or congestive heart failure. [i]Hypertension was defined as either a physician diagnosis, systolic blood pressure ≥140 mm/Hg, diastolic blood pressure ≥90 mm/Hg, or use of anti-hypertensive medications in the 30 days prior to enrollment. [j]Diabetes defined based on a physician diagnosis or use of any sugar-lowering medications in 30 days prior to enrollment. [k]Non-prescription and prescription non-steroidal anti-inflammatory drug (NSAID) use in the 30 days prior to blood draw.

Table 6.2 Multivariable-adjusted hazard ratios for cancer mortality by CMV IgG level, stratified by race, sex and cigarette smoking status, in CMV seropositive participants, ages 40 to 70 years, in the NHANES III study, 1988-2011

	Low CMV IgG	High CMV IgG
Total		
N	231	274
Person-Years	43734.1	42478.8
HR (95% CI)[a]	1.00 (Reference)	0.89 (0.67, 1.18)
Whites		
N	102	93
Person-Years	16192.9	14852.9
HR (95% CI)[a]	1.00 (Reference)	0.74 (0.53, 1.04)
Blacks		
N	63	115
Person-Years	11331	13811.2
HR (95% CI)[a]	1.00 (Reference)	1.38 (1.02, 1.89)*
Mexican Americans		
N	61	58
Person-Years	14475.8	12506.4
HR (95% CI)[a]	1.00 (Reference)	1.00 (0.60, 1.67)
Males		
N	133	139
Person-Years	23410.0	15907.5
HR (95% CI)[a]	1.00 (Reference)	1.00 (0.66, 1.50)
Females		
N	98	135
Person-Years	20324.2	25671.3
HR (95% CI)[a]	1.00 (Reference)	0.75 (0.52, 1.10)
Never Smokers		
N	72	67
Person-Years	18932.3	19046.2
HR (95% CI)[a]	1.00 (Reference)	0.80 (0.46, 1.42)
Former Smokers		
N	70	72
Person-Years	13542.9	11489.9
HR (95% CI)[a]	1.00 (Reference)	0.64 (0.33, 1.24)
Current Smokers		
N	89	135
Person-Years	11258.9	11942.7
HR (95% CI)[a]	1.00 (Reference)	1.12 (0.79, 1.58)

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Models adjusted for age, sex, race (non-Hispanic white, non-Hispanic black, Mexican American, other), education attainment (<high school diploma, high school diploma, > high school), marital status (married/cohabitating, widowed/separated/divorced, never married), census region (Northeast, Midwest, South, West), country of origin (U.S., foreign), annual family income (<$20,000, ≥$20,000), cigarette smoking status (never, former, current), pack-years (continuous), BMI (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m^2), health insurance coverage (yes/no), medical history (yes/no) of CVD and diabetes, NSAID use in past month (yes/no), and CRP level (<0.3, ≥0.3

Table 6.2 (continued) Multivariable-adjusted hazard ratios for cancer mortality by CMV IgG level, stratified by race, sex and cigarette smoking status, in CMV seropositive participants, ages 40 to 70 years, in the NHANES III study, 1989-2011

mg/dl). Asterisk indicates a significant interaction (p-interaction term=0.02) between black race, high CMV IgG level and white race low CMV IgG level.

Table 6.3 Multivariable-adjusted hazard ratios for cancer site-specific mortality by CMV IgG level in CMV seropositive participants, ages 40 to 70 years, in the NHANES III study, 1988-2011

	Low CMV IgG	High CMV IgG
Lung		
N	74	101
Person-Years	43734.1	42478.8
HR (95% CI)[a]	1.00 (Reference)	1.11 (0.70, 1.75)
Colorectal		
N	18	18
Person-Years	43734.1	42478.8
HR (95% CI)[a]	1.00 (Reference)	0.49 (0.15, 1.65)
Female Breast		
N	18	19
Person-Years	20324.2	26571.3
HR (95% CI)[b]	1.00 (Reference)	0.49 (0.17, 1.42)
Prostate		
N	15	19
Person-Years	15907.5	23410.0
HR (95% CI)[a]	1.00 (Reference)	1.44 (0.56, 3.73)

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Models adjusted for age, sex, race (non-Hispanic white, non-Hispanic black, Mexican American, other), education attainment (<high school diploma, high school diploma, > high school), marital status (married/cohabitating, widowed/separated/divorced, never married), census region (Northeast, Midwest, South, West), country of origin (U.S., foreign), annual family income (<$20,000, ≥$20,000), cigarette smoking status (never, former, current), pack-years (continuous), BMI (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m^2), health insurance coverage (yes/no), medical history (yes/no) of CVD and diabetes, NSAID use in past month (yes/no), and CRP level (<0.3, ≥0.3 mg/dl). [b]Additionally adjusted for menopausal status and hormone replacement therapy use (premenopausal, postmenopausal plus hormone use, postmenopausal and no hormone use).

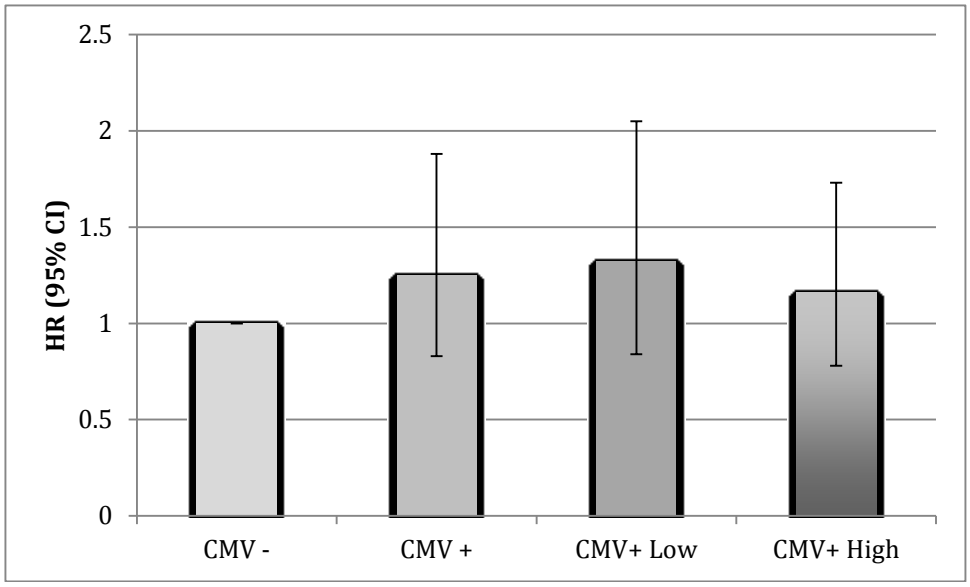

Figure 6.1 Multivariable-adjusted hazard ratios for cancer mortality by CMV serostatus and CMV IgG level in adults, ages 40 to 70 years, in the NHANES III study, 1988-2011. Models adjusted for age, sex, race (non-Hispanic white, non-Hispanic black, Mexican American, other), education attainment (<high school diploma, high school diploma, > high school), marital status (married/cohabitating, widowed/separated/divorced, never married), census region (Northeast, Midwest, South, West), country of origin (U.S., foreign), annual family income (<$20,000, ≥$20,000), cigarette smoking status (never, former, current), pack-years (continuous), BMI (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m^2), health insurance coverage (yes/no), medical history (yes/no) of CVD and diabetes, NSAID use in past month (yes/no), and CRP level (<0.3, ≥0.3 mg/dl).

Appendix D. Chapter 6 supplemental tables and figures

Table D.1 Baseline characteristics by levels of CMV IgG in white CMV seropositive adults, ages 40 to 70 years, in the NHANES III study, 1988-1994

	Low CMV IgG	High CMV IgG
Mean Age (years) (SE)	53.8 (0.42)	55.0 (0.50)
Male, %	53.7	37.1
Census Region, %		
Northeast	19.6	18.7
Midwest	25.2	25.5
South	38.1	39.0
West	17.0	16.8
Urban Residence[a], %	43.6	38.3
Country of Origin, %		
United States	93.3	92.1
Other	6.5	7.7
Missing	0.2	0.3
Household Crowding Index[b], %		
<0.5	61.0	62.7
0.5-0.99	33.9	33.9
≥1	5.1	3.4
Education, %		
Less than high school	35.5	40.4
High school diploma	23.9	26.4
More than high school	40.1	32.7
Missing	0.6	0.5
Marital Status, %		
Married, cohabitating	77.2	73.6
Widowed, divorced, separated	18.4	22.5
Never married	4.3	3.9
Missing	0.2	0.0
Annual Family Income ≥$20,000, %	76.2	67.6
Missing	0.7	1.7
Uninsured[c], %	7.8	9.4
Missing	1.3	1.3
≥1 Usual Source of Medical Care[d], %	85.2	86.7
Body Mass Index (kg/m^2)[e], %		
<18.5	1.1	2.1
18.5-24.9	34.0	32.1
25.0-29.9	37.5	35.7
30.0-34.9	18.5	18.4
≥35.0	8.9	11.7
Missing	0.2	0.0
Waist Circumference (cm), Mean (SE)	96.8 (0.72)	95.6 (0.5)
Missing, %	3.1	2.5
Postmenopausal[f], %	67.8	68.2
Cigarette Smoking Status, %		
Never	37.9	37.0
Former	37.7	33.9

Table D.1 (continued) Baseline characteristics by levels of CMV IgG in white CMV seropositive adults, ages 40 to 70 years, in the NHANES III study, 1988-1994

	Low CMV IgG	High CMV IgG
Current	24.5	29.0
Pack-Years[g], Mean (SE)	36.1 (1.5)	38.6 (1.4)
Missing, %	1.7	3.0
Cardiovascular Disease[h], %	6.9	9.0
Hypertension[i], %	44.9	43.8
Diabetes[j], %	5.9	7.7
Missing	0	0.3
C-Reactive Protein ≥0.3 mg/dL, %	33.2	35.5
Missing	0.2	1.0
NSAID Use in Past Month[k], %	63.0	62.3

Abbreviations: SE, standard error. [a]Urban residence defined as residing near a population of one million people based on the U.S. Department of Agriculture urban/rural code. [b]Household crowding index was calculated as the number of persons dwelling in a residence divided by the number of rooms. [c]Persons were uninsured if they did not have Medicare, Medicaid or private insurance. [d]Usual source of medical care was defined as having at least one medical facility or physician to contact in case of sickness or for routine care. [e]Body mass index calculated as weight (kg) divided by height (m) squared. [f]Postmenopausal was defined as not having a menstrual period in the past 12 months. [g]Pack-year calculated among ever smokers as the number of cigarettes per day multiplied by years smoking and then divided by 20. [h]Cardiovascular disease was defined as having had a heart attack, stroke, or congestive heart failure. [i]Hypertension was defined as either a physician diagnosis, systolic blood pressure ≥140 mm/Hg, diastolic blood pressure ≥90 mm/Hg, or use of anti-hypertensive medications in the 30 days prior to enrollment. [j]Diabetes defined based on a physician diagnosis or use of any sugar-lowering medications in 30 days prior to enrollment. [k]Non-prescription and prescription non-steroidal anti-inflammatory drug (NSAID) use in the 30 days prior to blood draw.

Table D.2 Baseline characteristics by levels of CMV IgG in black CMV seropositive adults, ages 40 to 70 years, in the NHANES III study, 1988-1994

	Low CMV IgG	High CMV IgG
Mean Age (years) (SE)	51.4 (0.48)	52.3 (0.39)
Male, %	51.0	36.0
Census Region, %		
Northeast	19.6	18.7
Midwest	25.2	25.5
South	38.1	39.0
West	17.0	16.8
Urban Residence[a], %	55.9	57.0
Country of Origin, %		
United States	91.5	92.8
Other	7.1	6.4
Missing	1.4	0.8
Household Crowding Index[b], %		
<0.5	45.6	41.1
0.5-0.99	38.5	40.8
≥1	15.9	18.1
Education, %		
Less than high school	33.5	34.9
High school diploma	36.8	43.4
More than high school	29.3	20.7
Missing	0.4	1.0
Marital Status, %		
Married, cohabitating	51.6	53.2
Widowed, divorced, separated	37.2	36.9
Never married	10.7	9.6
Missing	0.5	0.4
Annual Family Income ≥$20,000, %	52.0	44.6
Missing	1.7	3.5
Uninsured[c], %	14.3	14.9
Missing	1.1	1.0
≥1 Usual Source of Medical Care[d], %	82.6	82.4
Body Mass Index[e] (kg/m^2), %		
<18.5	2.6	1.9
18.5-24.9	28.3	24.3
25.0-29.9	35.7	35.7
30.0-34.9	18.8	21.9
≥35.0	14.4	16.2
Missing	0.2	0.0
Waist Circumference (cm), Mean (SE)	96.7 (0.8)	97.8 (0.5)
Missing, %	4.2	5.9
Postmenopausal[f], %	54.4	60.2
Cigarette Smoking Status, %		

Table D.2 (continued) Baseline characteristics by levels of CMV IgG in black CMV seropositive adults, ages 40 to 70 years, in the NHANES III study, 1988-1994

	Low CMV IgG	High CMV IgG
Never	41.1	40.9
Former	22.3	20.3
Current	36.6	38.8
Pack-Years[g], Mean (SE)	22.1 (0.9)	22.8 (0.8)
Missing, %	4.9	3.1
Cardiovascular Disease[h], %	8.1	10.2
Hypertension[i], %	56.9	59.8
Diabetes[j], %	10.2	13.1
Missing	0.0	0.1
C-Reactive Protein ≥0.3 mg/dL, %	41.4	49.4
Missing	1.0	1.9
NSAID Use in Past Month[k], %	38.2	44.3

Abbreviations: SE, standard error. [a]Urban residence defined as residing near a population of one million people based on the U.S. Department of Agriculture urban/rural code. [b]Household crowding index was calculated as the number of persons dwelling in a residence divided by the number of rooms. [c]Persons were uninsured if they did not have Medicare, Medicaid or private insurance. [d]Usual source of medical care was defined as having at least one medical facility or physician to contact in case of sickness or for routine care. [e]Body mass index calculated as weight (kg) divided by height (m) squared. [f]Postmenopausal was defined as not having a menstrual period in the past 12 months. [g]Pack-year calculated among ever smokers as the number of cigarettes per day multiplied by years smoking and then divided by 20. [h]Cardiovascular disease was defined as having had a heart attack, stroke, or congestive heart failure. [i]Hypertension was defined as either a physician diagnosis, systolic blood pressure ≥140 mm/Hg, diastolic blood pressure ≥90 mm/Hg, or use of anti-hypertensive medications in the 30 days prior to enrollment. [j]Diabetes defined based on a physician diagnosis or use of any sugar-lowering medications in 30 days prior to enrollment. [k]Non-prescription and prescription non-steroidal anti-inflammatory drug (NSAID) use in the 30 days prior to blood draw.

Table D.3 Baseline characteristics by levels of CMV IgG in Mexican American CMV seropositive adults, ages 40 to 70 years, in the NHANES III study, 1988-1994

	Low CMV IgG	High CMV IgG
Mean Age (years) (SE)	52.7 (0.96)	50.7 (0.72)
Male, %	53.0	38.6
Census Region, %		
Northeast	14.9	21.3
Midwest	8.1	10.7
South	39.1	31.6
West	38.0	36.4
Urban Residence[a], %	62.3	71.6
Country of Origin, %		
United States	34.7	33.8
Other	64.6	66.1
Missing	0.7	0.0
Household Crowding Index[b], %		
<0.5	22.1	24.2
0.5-0.99	47.4	49.1
≥1	30.2	26.6
Education, %	0.3	0.0
Less than high school	27.0	16.6
High school diploma	57.2	60.8
More than high school	14.2	22.3
Missing	1.6	2.8
Marital Status, %		
Married, cohabitating	77.3	69.4
Widowed, divorced, separated	16.8	23.5
Never married	5.2	7.0
Missing	0.6	0.0
Annual Family Income ≥$20,000, %	51.8	44.8
Missing	4.5	0.8
Uninsured[c], %	23.1	38.6
Missing	3.9	3.2
≥1 Usual Source of Medical Care[d], %	79.0	79.0
Body Mass Index[e] (kg/m^2), %		
<18.5	0.1	0.2
18.5-24.9	25.3	24.8
25.0-29.9	42.4	34.6
30.0-34.9	25.8	28
≥35.0	6.1	12.4
Missing	0.3	0.0
Waist Circumference (cm), Mean (SE)	96.7 (1.2)	97.1 (0.9)
Missing, %	2.5	4.2
Postmenopausal[f], %	59.3	50.5
Cigarette Smoking Status, %		

Table D.3 (continued) Baseline characteristics by levels of CMV IgG in Mexican American CMV seropositive adults, ages 40 to 70 years, in the NHANES III study, 1988-1994

	Low CMV IgG	High CMV IgG
Never	47.0	54.6
Former	31.5	21.0
Current	21.5	24.4
Pack-Years[g], Mean (SE)	22.7 (3.3)	16.9 (1.5)
Missing, %	1.3	2.1
Cardiovascular Disease[h], %	5.2	7.4
Hypertension[i], %	40.7	46.4
Diabetes[j], %	10.3	11.2
Missing	0.0	0.1
C-Reactive Protein ≥0.3 mg/dL, %	37.3	39.5
Missing	1.0	1.9
NSAID Use in Past Month[k], %	50.6	47.7

Abbreviations: SE, standard error. [a]Urban residence defined as residing near a population of one million people based on the U.S. Department of Agriculture urban/rural code. [b]Household crowding index was calculated as the number of persons dwelling in a residence divided by the number of rooms. [c]Persons were uninsured if they did not have Medicare, Medicaid or private insurance. [d]Usual source of medical care was defined as having at least one medical facility or physician to contact in case of sickness or for routine care. [e]Body mass index calculated as weight (kg) divided by height (m) squared. [f]Postmenopausal was defined as not having a menstrual period in the past 12 months. [g]Pack-year calculated among ever smokers as the number of cigarettes per day multiplied by years smoking and then divided by 20. [h]Cardiovascular disease was defined as having had a heart attack, stroke, or congestive heart failure. [i]Hypertension was defined as either a physician diagnosis, systolic blood pressure ≥140 mm/Hg, diastolic blood pressure ≥90 mm/Hg, or use of anti-hypertensive medications in the 30 days prior to enrollment. [j]Diabetes defined based on a physician diagnosis or use of any sugar-lowering medications in 30 days prior to enrollment. [k]Non-prescription and prescription non-steroidal anti-inflammatory drug (NSAID) use in the 30 days prior to blood draw.

Table D.4 Multivariable-adjusted hazard ratios for cancer mortality by CMV IgG level in black CMV seropositive participants, ages 40 to 70 years, in the NHANES III study, 1988-2011

	Low CMV IgG HR (95% CI)	**High CMV IgG** HR (95% CI)[a]
Excluding cancer deaths within 5 years of baseline	1.00 (Reference)	1.39 (1.02, 1.90)
Excluding users of prescription steroid drugs	1.00 (Reference)	1.41 (1.04, 1.93)
Excluding persons with a history of lupus at baseline	1.00 (Reference)	1.39 (1.02, 1.89)
Excluding persons with a history of rheumatoid arthritis at baseline	1.00 (Reference)	1.29 (0.89, 1.87)
Excluding baseline cases of skin cancer	1.00 (Reference)	1.39 (1.01, 1.89)

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Models adjusted for age, sex, race (non-Hispanic white, non-Hispanic black, Mexican American, other), education attainment (<high school diploma, high school diploma, > high school), marital status (married/cohabitating, widowed/separated/divorced, never married), census region (Northeast, Midwest, South, West), country of origin (U.S., foreign), annual family income (<$20,000, ≥$20,000), cigarette smoking status (never, former, current), pack-years (continuous), BMI (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m^2), health insurance coverage (yes/no), medical history (yes/no) of CVD and diabetes, NSAID use in past month (yes/no), and CRP level (<0.3, ≥0.3 mg/dl).

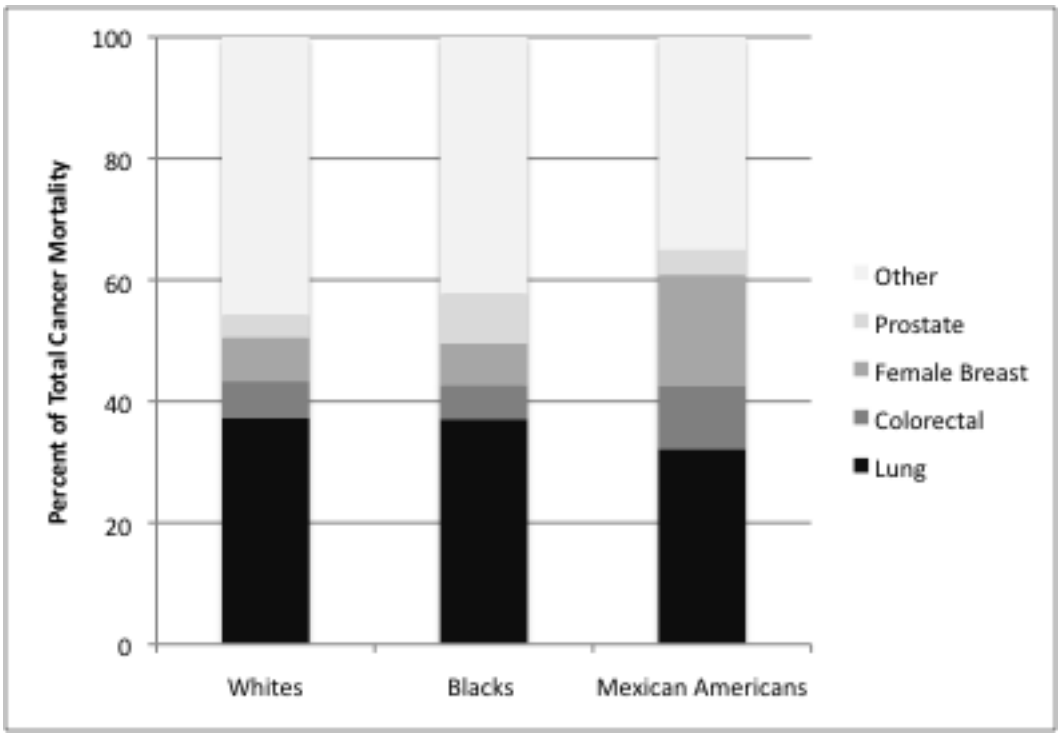

Figure D.1 Distribution of types of cancer mortality in NHANES III, 1988-2011

Table D.5 Multivariable-adjusted hazard ratios for cancer mortality by CMV serostatus and CMV IgG level, stratified by race, sex and cigarette smoking status, in participants, ages 40 to 70 years, in the NHANES III study, 1988-2011

		CMV Seropositive		
	CMV Seronegatives	**Overall**	**Low CMV IgG**	**High CMV IgG**
Total				
N	74	505	231	274
Person-Years	18572.3	86212.9	43734.1	42478.8
HR (95% CI)[a]	1.00 (Reference)	1.25 (0.83, 1.88)	1.32 (0.84, 2.09)	1.16 (0.78, 1.73)
P-Trend				0.63
Whites				
N	58	195	102	93
Person-Years	14180.8	31045.8	16192.9	14852.9
HR (95% CI)[a]	1.00 (Reference)	1.24 (0.79, 1.94)	1.41 (0.85, 2.35)	1.05 (0.68, 1.61)
P-Trend				0.96
Blacks				
N	11	178	63	115
Person-Years	2242.6	25142.2	11331.0	13811.2
HR (95% CI)[a]	1.00 (Reference)	1.64 (0.92, 2.90)	1.35 (0.71, 2.57)	1.89 (1.09, 3.30)
P-Trend				0.003
Mexican Americans				
N	4	119	61	58
Person-Years	1936.8	26982.2	14475.8	12506.4
HR (95% CI)[a]	1.00 (Reference)	3.20 (0.60, 17.0)	3.19 (0.58, 17.6)	3.23 (0.62, 16.89)
P-Trend				0.24
Males				
N	49	272	133	139
Person-Years	10416.6	39317.5	23410.0	15907.5
HR (95% CI)[a]	1.00 (Reference)	1.29 (0.80, 2.08)	1.31 (0.74, 2.30)	1.27 (0.82, 1.95)
P-Trend				0.3

Table D.5 (continued) Multivariable-adjusted hazard ratios for cancer mortality by CMV serostatus and CMV IgG level, stratified by race, sex and cigarette smoking status, in participants, ages 40 to 70 years, in the NHANES III study, 1988-2011

	CMV Seronegatives	CMV Seropositive		
		Overall	Low CMV IgG	High CMV IgG
Females				
N	25	233	98	135
Person-Years	8155.7	46895.5	20324.2	26571.3
HR (95% CI)[a]	1.00 (Reference)	1.19 (0.65, 2.17)	1.37 (0.73, 2.59)	1.04 (0.55, 1.97)
P-Trend				0.77
Never Smokers				
N	17	139	72	67
Person-Years	7836.0	37978.5	18932.3	19046.2
HR (95% CI)[a]	1.00 (Reference)	1.23 (0.50, 3.04)	1.37 (0.58, 3.28)	1.06 (0.38, 3.00)
P-Trend				0.92
Former Smokers				
N	30	142	70	72
Person-Years	6450.2	25032.8	13542.9	11489.9
HR (95% CI)[a]	1.00 (Reference)	1.25 (0.66, 2.39)	1.50 (0.70, 3.21)	0.95 (0.51, 1.77)
P-Trend				0.73
Current Smokers				
N	27	224	89	135
Person-Years	4286.2	23201.6	11258.9	11942.7
HR (95% CI)[a]	1.00 (Reference)	1.39 (0.78, 2.50)	1.34 (0.79, 2.26)	1.45 (0.74, 2.85)
P-Trend				0.29

Abbreviations: HR, hazard ratio; CI, confidence interval. [a]Models adjusted for age, sex, race (non-Hispanic white, non-Hispanic black, Mexican American, other), education attainment (<high school diploma, high school diploma, > high school), marital status (married/cohabitating, widowed/separated/divorced, never married), census region (Northeast, Midwest, South, West), country of origin (U.S., foreign), annual family income (<$20,000, ≥$20,000), cigarette smoking status (never, former, current), pack-years (continuous), BMI (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, ≥35.0 kg/m^2), health insurance coverage (yes/no), medical history (yes/no) of CVD and diabetes, NSAID use in past month (yes/no), and CRP level (<0.3, ≥0.3 mg/dl)

Chapter 7. Conclusion

The primary objective of this dissertation was to investigate the effects of the immune states, low-grade chronic inflammation and subclinical immunosuppression, on the risk of cancer incidence and mortality. This dissertation work complements rapidly accumulating data from laboratory studies elucidating the role of immune components in carcinogenesis by quantifying the risk of cancer incidence and mortality by levels of immune markers in immunocompetent adults. Additionally, we explored socio-demographic, behavioral, and genetic factors that may modify the effects of these factors on carcinogenesis.

In order to study the complex roles of host immunity in carcinogenesis, we utilized the immune markers, white blood cell (WBC) subtype (i.e., neutrophil, lymphocyte, monocyte, basophil) count and cytomegalovirus (CMV) IgG antibody titer. Based on studies conducted in the laboratory and clinical setting, each of the WBC subtypes have differential roles in host immunity and may also exert unique effects in carcinogenesis (1, 2). Additionally, CMV is postulated to be a surrogate marker and/or causal factor of suppressed immunity and deregulated inflammation; this may be an important mechanism linking CMV infection with cancer (3). Furthermore, in the interest of evaluating the effects of low-grade inflammation and subclinical immune capacity, specifically, we examined inter-individual variations of these markers among immunocompetent adults, restricting the analytic cohorts to persons with total WBC count within the normal reference range, in analyses of WBC subtype counts, and persons with asymptomatic CMV infection.

In chapters two through five of this dissertation, we present our findings from a prospective study conducted in the Atherosclerosis Risk in Communities (ARIC) study on the relationship between pre-diagnostic WBC counts and cancer incidence and mortality. These findings are summarized in Table 7.1. Among men and women with total WBC counts within the normal reference range, higher neutrophil count was associated with an 11% increased risk of total cancer incidence and a 44% increased risk of total cancer mortality. These positive associations were independent of the effects of the other WBC subtypes and in cancer site-specific analyses higher neutrophil count was associated with significant increased risks of lung cancer incidence and mortality and breast cancer mortality.

We also found that men with higher lymphocyte counts had a 25% reduced risk of cancer incidence, after excluding cases of prostate cancer, and an increased risk of prostate cancer incidence. Similar significant findings were present after mutual adjustment for the other WBC subtypes. In contrast, in women, high lymphocyte count was associated with a 40% *increased* risk of cancer mortality. However, in stratified analyses, this association was only present among current smokers; thus, the independent contribution of lymphocytes to cancer mortality in women is questionable.

Although basophils constitute a very small proportion of the total WBC count (0-3%), we found that the presence of these cells in the peripheral blood was associated with a reduced risk of cancer incidence and mortality. Notably, this association was independent

of the other WBC subtypes, including eosinophil count, another WBC subtype also involved in the host allergic response.

High monocyte count was associated with a slight, but not significant, increased risk of total cancer mortality and a significant increased risk of lung cancer mortality. However, after further adjustment for the other WBC subtypes, these associations were no longer found. Furthermore, in comparisons of monocyte count measured three years apart, there was considerable intra-individual variation, suggesting that this marker may not be informative in either the research or clinical settings.

Lastly, chapter six examines the prospective association between CMV IgG level and cancer mortality among CMV seropositive men and women, ages 40-70, in the third National Health and Nutrition Examination Survey (NHANES III). In this study, higher CMV IgG antibody titer was associated with a 38% increased risk of cancer mortality in blacks who are CMV seropositive, while no association was present in whites or Mexican Americans.

To our knowledge, this dissertation is among the first efforts to thoroughly evaluate the association between pre-diagnostic WBC subtype counts and CMV IgG antibody titer and subsequent cancer incidence and mortality. Generally, our findings suggest that low-grade inflammation and subclinical immunosuppression, as measured by these circulating immune markers, may be associated with an increased risk of cancer incidence and

mortality. Furthermore, these findings may have important implications for both cancer etiology and cancer prevention, as described below.

While previous epidemiological studies have examined total WBC count as a marker of systemic inflammation in the context of tumor development and progression (4-8), we provide evidence that each of the WBC subtypes may be involved in different, potentially opposing, etiological pathways in carcinogenesis. In particular, high neutrophil count, within the normal reference range, may be a relevant marker of the pro-tumoral effects of chronic inflammation. Our findings expand upon laboratory studies, which have shown that neutrophils in the tumor microenvironment may directly promote tumor progression (9, 10), by suggesting that pre-diagnostic neutrophil count, measured years prior to the onset of cancer, may also be relevant to subsequent cancer incidence and mortality. This is a particularly important contribution to the cancer etiology literature as in vitro models of inflammation in the early stages of carcinogenesis are missing (1). Notably, although macrohages are also a critical component of the tumor micro-environment (11), pre-diagnostic monocytes, a precursor of macrophages, does not seem to be a marker of cancer risk. This finding, however, does not preclude the possibility that the pre-diagnostic macrophage-mediated inflammatory response in the tissue may be relevant in carcinogenesis.

In addition to the pro-tumoral effects of chronic inflammation, the host immune response may also exert anti-tumoral effects. The tumor immunosurveillance hypothesis, which posits a role for the host immune system in identifying and clearing premalignant and

malignant cells, was introduced over 50 years ago (12). However, this hypothesis has garnered more wide-spread interest only recently based on accumulating experimental evidence from mice models and prognostic studies (13). Our findings of an inverse association between lymphocyte count and cancer incidence in men and an inverse association between basophils and cancer incidence and mortality, overall, provide some of the first epidemiological support for this concept. Moreover, our findings suggest that even normal variations in immune capacity, as measured by counts of these markers, may affect the tumor immunosurveillance response.

Our findings may also support the direct involvement of lymphocytes and basophils in the tumor immunosurveillance response. Additionally, we observed a differential association between lymphocyte count and cancer by sex, such that an inverse association with cancer incidence was present in men only and a positive association with cancer mortality was found in women. Moreover, among men, the inverse association with cancer incidence was limited to non-prostate cases of cancer. These findings suggest, for the first time, that the role of lymphocytes in carcinogenesis may be dependent on the hormonal milieu. More specifically, it is plausible that hormonal levels affect the absolute and relative levels of circulating and localized lymphocyte subsets (14), accounting for our study findings.

Historically, basophils have not been a well-studied component of the immune system in part because of the low levels of basophils in healthy individuals. Our findings of an inverse association between basophil count and cancer incidence and mortality may

suggest a novel role for this cell as part of the tumor immunosurveillance response. Indeed, there are several potential pathways by which basophils may exert anti-tumoral effects, including through the production of histamine and interleukin-4, both of which have anti-tumoral properties (15-17).

Cytomegalovirus activity may be another informative marker of host immunity in the context of cancer development and progression. Specifically our findings suggest that the host immune response to CMV infection, as measured by CMV IgG antibody titer, may be a marker of cancer mortality risk among black men and women. While our findings may suggest a potential direct role for CMV in carcinogenesis, possibly through the suppression of the immune response and the deregulation of inflammation, we cannot discount alternate possible explanations. For example, CMV activity may be a surrogate marker of host immune capacity or another, unmeasured factor. Furthermore, our finding of a differential association by race may also have important implications towards explaining and reducing the disparate burden of cancer deaths in blacks.

The results presented in this dissertation may also have important future implications for the clinical setting. Circulating levels of neutrophils, lymphocytes, and basophils may have utility for cancer risk stratification in the general immunocompetent population. In particular, combining these markers into a panel may be most appropriate as we have shown that each of these factors have independent effects on the risk of cancer incidence and mortality. More refined risk stratification, in turn, may guide clinical decision-making in terms of screening recommendations, the promotion of behavior modifications,

and the use of preventative agents, which may reduce inflammation and increase immune capacity. Importantly, we have shown that many modifiable lifestyle factors are associated with differences in levels of these immune markers within the normal reference range. Pending validation of the predictability of these markers, integrating these measures into clinical practice would likely be feasible as the measurement of WBC subtype counts is non-invasive, inexpensive, and frequently recommended for other indications.

Additionally, if future studies support a causal role for the WBC subtypes and CMV in carcinogenesis, the development of interventions targeting specific immune pathways may also be a beneficial preventative strategy. While substantial efforts have been dedicated to the development of immunotherapeutic approaches for cancer treatment (18), similar approaches may be applicable in the preventative setting.

The significance of this area of research, more generally, is underscored by its widespread applicability. First, the immune states, low-grade inflammation and immunosupression, are hypothesized to be shared etiological factors for many commonly occurring cancers (1, 19). Furthermore, in addition to being independent etiological factors in carcinogenesis, these immune pathways are also hypothesized to mediate the effects of many common exposures, such as obesity, cigarette smoke, and age (1). Finally, in this study, we demonstrate that elevated levels even within the normal reference range may be meaningful in terms of cancer incidence and mortality risk. Thus,

a substantial proportion of the burden of cancer incidence and mortality in U.S. adults may be explained by immune factors.

Perhaps the greatest challenge in the interpretation our study findings is the use of a one-time measure of WBC subtype count and CMV IgG level. In these analyses, we assume that a one-time measure of these biomarkers reflects the usual baseline immune response in healthy adults. In order to strengthen the basis of this assumption, in analyses of WBC subtype count we restricted the analytic cohort to persons with total WBC counts within the clinically normal range so as not to include episodes of acute immune response. Additionally, in sensitivity analyses we further excluded persons with specific WBC subtype counts outside the normal reference range.

While these approaches may minimize the likelihood of including acute responses, they do not account for intra-individual variation within the normal reference range. To our knowledge, the short- and long-term variation of WBC subtypes has not been previously investigated. However, in a study with over 40 years of follow-up, accounting for time-varying measures of total WBC count measured every two years compared to using a single baseline measure did not markedly alter the association between total WBC count and all cause mortality (20). Additionally, in comparisons of WBC subtype count measured at baseline and three years later, in ARIC, we found that there was fair agreement for each of the WBC subtypes, with the exception of monocyte count. Notably, however, the consequences of intra-individual variation in the range observed on the attenuation of risk estimates has been shown to be substantial for moderate

associations (21). Thus, our findings are likely underestimates of the true associations. With respect to CMV IgG levels, there is no longitudinal data evaluating changes over time. However, by categorizing CMV IgG levels broadly, we minimize the effects of intra-individual changes over time. Future studies will be necessary to determine if our findings persist after accounting for time-varying changes of both WBC subtype counts and CMV IgG and to explore different relevant periods of exposure over the lifetime.

Pre-diagnostic levels of WBC subtype count and CMV IgG may influence carcinogenesis at various stages, from tumor initiation to tumor progression and metastasis. In the absence of information on tumor stage and grade at diagnosis and the treatments administered, however, we were unable to determine at what stage of carcinogenesis these markers may be most relevant. Nevertheless, in analyses of WBC subtype count, we noted differences in the risk of cancer incidence versus mortality suggesting that the pathways mediated by neutrophils and basophils may be more relevant for tumor progression while lymphocytes may be primarily involved in the earliest stages of carcinogenesis, leading to tumor initiation. In contrast, in analyses of CMV IgG, information on cancer incidence was missing, thus we were only able to evaluate the association of this marker with a unique subset of cancers that progress to metastasis and death.

Despite these limitations, this dissertation also has several strengths. We utilized data collected in two large and well-established studies, ARIC and NHANES III. In these studies, information on cancer incidence (ARIC only) and cancer mortality was highly

complete, based primarily on cancer registry data and the National Death Index (NDI), and was prospectively collected over 17 to 23 years of follow-up. Few other studies with prospectively collected data on cancer outcomes have also measured baseline immune markers. Additionally, at baseline, information on relevant, potential confounders, including education attainment, cigarette smoking status, pack-years, alcohol intake, body mass index (BMI), non-steroidal anti-inflammatory drug (NSAID) use and medical history of cardiovascular disease (CVD), diabetes and hypertension, were collected using standardized approaches. Another strength of these studies is the large sample size, which included over 10,000 participants in ARIC and 5,000 participants in NHANES III. Given the large sample sizes, we were able to explore effect modification by several important factors, including sex, BMI category, and cigarette smoking status and to examine cancer site-specific analyses. Further, in both study populations, racial minorities were well represented enabling us to explore differences by race.

Given that the studies included in this dissertation are among the first to examine the associations between pre-diagnostic WBC subtype and CMV IgG level and cancer incidence and/or mortality, we undertook a broad approach to examining these relationships. As such, our findings should be considered exploratory and hypothesis generating. Future studies are warranted to validate our results and to expand upon our findings.

Our findings point to several potential directions for future research. Firstly, larger studies are necessary to explore the association between these immune markers and

cancer site-specific incidence and mortality. Such studies will provide more accurate estimates of the risk associated with these immune markers and identify potential differential associations by cancer site. Because WBC subtype count can only be measured within 24 hours of blood draw, however, it will be challenging to identify large cohort studies with prospectively collected information on cancer outcomes, which have already measured subtype counts. Identifying large studies with available measures of CMV IgG may be more feasible as this titer can be measured in stored blood samples in established cohorts.

Secondly, in order to explore the potential etiological pathways mediated by WBC subtypes and CMV IgG, measurements of subpopulations of WBC subtypes and additional immune markers may be informative. In particular, information on the relative and absolute counts of lymphocyte subsets may clarify our finding of a differential role for lymphocytes in carcinogenesis in men and women, and including downstream products of neutrophils, lymphocytes and basophils into models may also provide insight into these etiological pathways. Additionally, one proposed mechanism linking CMV infection to tumor development and progression is through the deregulation of inflammatory pathways and the induction of immune suppression. In order to evaluate the relevance of this pathway in immunocompetent adults, it will be necessary to quantify to what extent markers of inflammation and immune capacity mediate the effects of this virus on cancer incidence and mortality.

Thirdly, large networks of genes affect immune function and functional polymorphisms of these genes have been associated with several types of cancers (22-27). Indeed, several genetic variants associated with WBC subtype phenotype and CMV pathogenesis have been identified (28-31). Exploring the association between these variants and cancer may elucidate the etiological role of these immune factors. Furthermore, such analyses may be preferable to evaluating circulating levels of markers, as genetic variants are not subject to confounding or the effects of subclinical disease (32). Genetic variants may also modify the association between circulating levels of immune markers and cancer risk. As part of this dissertation, we considered the effects of a single nucleotide polymorphism (SNP) in the gene for the Duffy antigen receptor for chemokines (DARC) in analyses of neutrophils count. While the presence of this polymorphism did not significantly modify the association between neutrophil count and subsequent cancer incidence and mortality, there was a suggestive interaction suggesting that among carriers of this polymorphism neutrophil count may be more strongly associated with cancer mortality. Genetic analyses may be particularly informative in future analyses examining CMV IgG levels as genetic differences may explain our finding of a differential association by race.

Lastly, studies evaluating the predictive accuracy of these immune biomarkers in addition to traditional risk factors for subsequent cancer risk and progression will be necessary to determine the clinical significance of our findings. While, estimating the c statistic using receiver operating characteristic (ROC) curves may help to address the future clinical utility of these markers, comparisons of global model fit based on the log likelihood function may also provide important information (33).

References

1. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140:883-99.
2. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420:860-7.
3. Soderberg-Naucler C. Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer? J Intern Med. 2006;259:219-46.
4. Margolis KL, Rodabough RJ, Thomson CA, Lopez AM, McTiernan A. Prospective study of leukocyte count as a predictor of incident breast, colorectal, endometrial, and lung cancer and mortality in postmenopausal women. Arch Intern Med. 2007;167:1837-44.
5. Erlinger TP, Muntner P, Helzlsouer KJ. WBC count and the risk of cancer mortality in a national sample of U.S. adults: results from the Second National Health and Nutrition Examination Survey mortality study. Cancer Epidemiol Biomarkers Prev. 2004;13:1052-6.
6. Prizment AE, Anderson KE, Visvanathan K, Folsom AR. Association of inflammatory markers with colorectal cancer incidence in the atherosclerosis risk in communities study. Cancer Epidemiol Biomarkers Prev. 2011;20:297-307.
7. Shankar A, Wang JJ, Rochtchina E, Yu MC, Kefford R, Mitchell P. Association between circulating white blood cell count and cancer mortality: a population-based cohort study. Arch Intern Med. 2006;166:188-94.
8. Godsland IF, North BV, Johnston DG. Simple indices of inflammation as predictors of death from cancer or cardiovascular disease in a prospective cohort after two decades of follow-up. QJM. 2010.
9. Brandau S, Dumitru CA, Lang S. Protumor and antitumor functions of neutrophil granulocytes. Semin Immunopathol. 2013;35:163-76.
10. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. Cancer Cell. 2009;16:183-94.
11. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454:436-44.
12. Burnet M. Cancer: a biological approach. III. Viruses associated with neoplastic conditions. IV. Practical applications. Br Med J. 1957;1:841-7.
13. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Adv Immunol. 2006;90:1-50.
14. Olsson J, Wikby A, Johansson B, Lofgren S, Nilsson BO, Ferguson FG. Age-related change in peripheral blood T-lymphocyte subpopulations and cytomegalovirus infection in the very old: the Swedish longitudinal OCTO immune study. Mech Ageing Dev. 2000;121:187-201.
15. Stanciu L. Immunomodulation by histamine. Ann Biol Clin (Paris). 1990;48:623-5.
16. Schroeder JT, MacGlashan DW, Jr., Lichtenstein LM. Human basophils: mediator release and cytokine production. Adv Immunol. 2001;77:93-122.

17. Voehringer D, Shinkai K, Locksley RM. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. Immunity. 2004;20:267-77.
18. Harris TJ, Drake CG. Primer on tumor immunology and cancer immunotherapy. J Immunother Cancer. 2013;1:12.
19. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell. 2005;7:211-7.
20. Ruggiero C, Metter EJ, Cherubini A, Maggio M, Sen R, Najjar SS, et al. White blood cell count and mortality in the Baltimore Longitudinal Study of Aging. J Am Coll Cardiol. 2007;49:1841-50.
21. Platz EA, Sutcliffe S, De Marzo AM, Drake CG, Rifai N, Hsing AW, et al. Intra-individual variation in serum C-reactive protein over 4 years: an implication for epidemiologic studies. Cancer Causes Control. 2010;21:847-51.
22. Sun T, Hu Z, Shen H, Lin D. Genetic polymorphisms in cytotoxic T-lymphocyte antigen 4 and cancer: the dialectical nature of subtle human immune dysregulation. Cancer Res. 2009;69:6011-4.
23. Oh BR, Sasaki M, Perinchery G, Ryu SB, Park YI, Carroll P, et al. Frequent genotype changes at -308, and 488 regions of the tumor necrosis factor-alpha (TNF-alpha) gene in patients with prostate cancer. J Urol. 2000;163:1584-7.
24. Srivastava K, Srivastava A, Kumar A, Mittal B. Significant association between toll-like receptor gene polymorphisms and gallbladder cancer. Liver Int. 2010;30:1067-72.
25. Shiels MS, Engels EA, Shi J, Landi MT, Albanes D, Chatterjee N, et al. Genetic variation in innate immunity and inflammation pathways associated with lung cancer risk. Cancer. 2012;118:5630-6.
26. Gong Z, Quan L, Yao S, Zirpoli G, Bandera EV, Roberts M, et al. Innate immunity pathways and breast cancer Risk in African American and European-American women in the Women's Circle of Health Study (WCHS). PLoS One. 2013;8:e72619.
27. Brenner AV, Neta G, Sturgis EM, Pfeiffer RM, Hutchinson A, Yeager M, et al. Common single nucleotide polymorphisms in genes related to immune function and risk of papillary thyroid cancer. PLoS One. 2013;8:e57243.
28. Nalls MA, Couper DJ, Tanaka T, van Rooij FJ, Chen MH, Smith AV, et al. Multiple loci are associated with white blood cell phenotypes. PLoS Genet. 2011;7:e1002113.
29. Kang SH, Abdel-Massih RC, Brown RA, Dierkhising RA, Kremers WK, Razonable RR. Homozygosity for the toll-like receptor 2 R753Q single-nucleotide polymorphism is a risk factor for cytomegalovirus disease after liver transplantation. J Infect Dis. 2012;205:639-46.
30. Kijpittayarit S, Eid AJ, Brown RA, Paya CV, Razonable RR. Relationship between Toll-like receptor 2 polymorphism and cytomegalovirus disease after liver transplantation. Clin Infect Dis. 2007;44:1315-20.
31. Nahum A, Dadi H, Bates A, Roifman CM. The biological significance of TLR3 variant, L412F, in conferring susceptibility to cutaneous candidiasis, CMV and autoimmunity. Autoimmun Rev. 2012;11:341-7.
32. Prizment AE, Folsom AR, Dreyfus J, Anderson KE, Visvanathan K, Joshu CE, et al. Plasma C-reactive protein, genetic risk score, and risk of common cancers in the Atherosclerosis Risk in Communities study. Cancer Causes Control. 2013;24:2077-87.

33. Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation. 2007;115:928-35.

	Cancer Incidence	Cancer Mortality
Neutrophils (Tertile 3/Tertile 1)	↑ Total and lung cancer-specific; among current smokers only	↑ Total, lung and breast cancer; among current, former and never smokers
Lymphocytes (Tertile 3/Tertile 1)	↓ Among men, excluding cases of prostate cancer; current, former and never smokers ↑ Prostate cancer incidence	↑ Among female current smokers only
Basophils (presence/absence)	↓ Suggestive association with total cancer	↓ Total and lung cancer; among never smokers
Monocytes (Tertile 3/Tertile 1)	No association	No association

Table 7.1 Summary of the independent associations between pre-diagnostic WBC subtype counts and subsequent cancer incidence and mortality in the ARIC study.

CURRICULUM VITAE

Sarah Alice Davidovics, ScM

Personal Information

2708 Jenner Drive, Apt. E
Baltimore, MD 21209
Mobile: (206) 947-9468
Email: sisrael2@jhu.edu

Education

Doctorate of Philosophy (PhD) candidate, Epidemiology
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Dissertation Title: *Low-grade inflammation, immune capacity, and cancer*

2009 **Master of Science (ScM)**, Epidemiology
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Thesis Title: *Plasma cholesterol and the risk of benign breast disease: A prospective study of women in Washington County, MD*

2005 **Bachelor of Arts (BA)**, Biology (Minor: Political Science), summa cum laude
Barnard College, Columbia University, New York, NY

Research Experience

2008-2014 **Senior Research Assistant**, Department of Epidemiology, Bloomberg School of Public Health, Baltimore, MD. Mentor: Dr. Kala Visvanathan

Developed study design, performed statistical analyses, and prepared manuscripts and grants for a large cohort study. Other responsibilities included creating and managing databases, questionnaires, and study protocols. Research areas: BRCA1/2 mutation carriers, molecular markers of early detection, benign breast disease, immunity and cancer.

2006-2007 **Molecular Diagnostics Laboratory Aide**, Fred Hutchinson Cancer Research Center, Seattle, WA. Mentor: Paul Lampe

Maintained tissue cultures of 30 mammalian cell lines and routinely conducted western-blot analysis under varying conditions.

Teaching Positions

2012-2014 Guest lecturer, Clinical Aspects of Reproductive Health
Department of Population, Family and Reproductive Health
Bloomberg School of Public Health, Baltimore, MD

2012 Teaching Assistant, Epidemological Methods I
Department of Epidemiology
Bloomberg School of Public Health, Baltimore, MD

2009 Teaching Assistant, Cancer Etiology, Prevention, and Control
Department of Epidemiology
Bloomberg School of Public Health, Baltimore, MD

2008 Teaching Assistant, Principles of Epidemiology I
Department of Epidemiology
Bloomberg School of Public Health, Baltimore, MD

2008 Teaching Assistant, Fundamentals of Epidemiology
Department of Public Health Studies
Johns Hopkins Krieger School of Arts and Sciences, Baltimore, MD

Honors and Awards

2010-2014 NIH, National Cancer Institute, Cancer Epidemiology, Prevention, and Control T32 training grant

2013 Third prize in Clinical/Translational Research section at the Sydney Kimmel Comprehensive Cancer Center at Johns Hopkins Breast Cancer Retreat Poster Session

2011 Harvey Meyerhoff Award, Department of Epidemiology, Bloomberg School of Public Health

2010 First prize in Clinical/Translational Research section at the Sydney Kimmel Comprehensive Cancer Center at Johns Hopkins Breast Cancer Retreat Poster Session

2009 Trudy Bush Fund, Bloomberg School of Public Health

2007 Offered Yale School of Public Health Dean's Scholarship

2005 Phi Beta Kappa, Barnard College

2005 Biology Department Honors, Barnard College

2005 Senior Thesis Distinction, Barnard College

2004 Howard Hughes Research Fellow, Barnard College

Academic Activities

2011-2013 Member of American Association of Cancer Research

2008-2009 Coordinator of Cancer Epidemiology, Prevention, and Control Journal Club

Skills

Word, Power Point, Excel, Microsoft Access, Stata, SAS, R

Presentations/Publications

Gross AL, **Davidovics SA,** Axilbund JE, Armstrong DK, May BJ, Visvanathan K. Longitudinal comparison of body mass index (BMI) in breast cancer survivors to cancer-free women: A prospective study in high-risk women [poster]. In: ASCO Annual Meeting, July 2013. *J Clin Oncol* 31, 2013 (suppl; abstr 1601).

Sarah A. Davidovics, Anna Prizment, Kumar Visvanathan, Kala Visvanathan. Neutrophil count, cancer incidence and cancer mortality: disparate relationships by race. [abstract]. In: Proceedings of the 104th Annual Meeting of the American Association for Cancer Research; 2013 Apr 6-10; Washington, DC. Philadelphia (PA): AACR; Cancer Res 2013;73(8 Suppl:Abstract nr 2525).

S.A. Israel, H. Warzecha, A.L. Gross, A. Tatsas, A. Lee, B. May, J. Axilbund, N. Khouri, L. Jacobs, D. Armstrong, K. Visvanathan. Progression from benign breast disease to cancer in women with a family history of breast cancer [poster]. In: Proceedings of the 103rd Annual Meeting of the American Association for Cancer Research; 2012 Mar 31-Apr 4; Chicago, IL. Philadelphia (PA): AACR; Cancer Res 2012;72(8 Suppl:Abstract nr 3568).

S.A. Israel, H. Nassar, A.L. Gross, L.K. Jacobs, D.K. Armstrong, K. Visvanathan. Characterizing benign breast disease in women at high risk for breast cancer [oral abstract]. In: ASCO; June 2010. J Clin Oncol 28:15s, 2010 (suppl; abstr 1506).

Contributor: U.S. Department of Health and Human Services (2014). The Health Consequences of Smoking – 50 Years of Progress: A Report of the Surgeon General.